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(54) **Title**: LAMIN A, AN ACTIVATOR OF LONGEVITY/ANTI-AGING SIRT1 PROTEIN

(57) **Abstract**: The present invention provides methods of modulating the deacetylase activity of SIRT1 by modifying the binding affinity of lamin Ato SIRT1 via interaction modifying compound. The present invention also provides methods of screening SIRT1 modulating compounds based on the interaction between lamin A and SIRT1 protein and SIRT1-activating property of lamin A. The present invention further discloses uses of SIRT1 -activating compounds to treat patients suffering from metabolic and/or aging-related degenerative diseases, and uses of SIRT1 -inhibiting compounds to treat human malignancies.

DESCRIPTION

LAMIN A, AN ACTIVATOR OF LONGEVITY/ANTI-AGING SIRT1 PROTEIN

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. provisional application Serial No. 61/725,252, filed November 12, 2012, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

Sirtuins (silent mating type information regulation 2 homolog), class III HDACs containing NAD^+-dependent protein deacetylase and ADP-ribosyltransferase activities, regulate various metabolic pathways (Denu, 2005; Donmez and Guarente, 2010; Finkel et al., 2009; Haigis and Sinclair, 2010).

Of seven mammalian sirtuins, SIRT1 (NAD-dependent deacetylase sirtuin-1) is the closest homolog of *Saccharomyces cerevesiae* Sir2 (silent information regulator 2) identified three decades ago (Klar et al., 1979). Loss of SIRT1 causes defective gametogenesis, heart and retinal abnormalities, genomic instability, small body size, and reduced survival in mice (Cheng et al., 2003; McBurney et al., 2003; Wang et al., 2008); and abolishes many beneficial effects of dietary restriction (Chen et al., 2005). Although lifespan extension in *Saccharomyces cerevesiae, C. elegans and Drosophila* by ectopic Sir2 is still under debate (Burnett et al., 2011; Lombard et al., 2011; Tissenbaum and Guarente, 2001; Viswanathan and Guarente, 2011; Viswanathan et al., 2005), transgenic mice with additional copies of SIRT1 show phenotypes resembling dietary restriction and consistent with improved healthspan (Alcendor et al., 2007; Banks et al., 2008; Bordone et al., 2007; Herranz et al., 2010; Pfluger et al., 2008).

SIRT1 deacetylates a variety of proteins, including KU70, Nbs1, p53, NF-κB, PPARγ, PGC-1α, FOXO, and SUV39H1, and regulates genomic integrity, the inflammatory response, adipogenesis, mitochondrial biogenesis, and stress resistance (Lavu et al., 2008). For example,

SIRT1 catalyzes the deacetylation of tumor suppressor protein p53, thus promoting survival by inhibiting p53-mediated apoptosis (Cheng et al., 2003). SIRT1 also directly interacts with PPAR-γ and PGC-1α, thus regulating metabolic response (Picard et al., 2004; Rodgers et al., 2005).

In addition, SIRT1 deacetylates Foxo3a to enhance stress resistance through Foxo3a targets such as MnSOD, catalase, and Gadd45α (Brunet et al., 2004). SIRT1 is highly expressed in embryonic stem cells (ESCs), but its expression is reduced in differentiated cells through a process mediated by miRNAs (Saunders et al., 2010). SIRT1 is required for maintenance of self-renewal of ESCs via modulating p53 cellular distribution and Nanog expression (Han et al., 2008). The hematopoietic differentiation of ESCs is defective and the number and function of hematopoietic progenitor cells decline in *SIRT1*$^{-/-}$ and *SIRT1*$^{+/-}$ mice (Lee et al., 2011). When cultured under 5% oxygen, both *SIRT1*$^{-/-}$ and *SIRT1*$^{+/-}$ hematopoietic progenitor cells exhibit defective proliferation compared with wild-type cells (Mantel et al., 2008).

SIRT1 is one of the most conserved anti-aging/longevity-promoting proteins across species. Increase in SIRT1 deacetylase activity confers many beneficial effects on various mouse models mimicking human metabolic or degenerative diseases, such as obesity, diabetes, and Alzheimer's Diseases. Therefore, SIRT1-activating compounds could benefit human patients suffering from various metabolic and aging-related degenerative diseases. On the other hand, SIRT1 protein is found upregulated in various human cancers, and inhibition of SIRT1 activity could help in eliminating cancer stem cells (Li et al., 2012).

Increased SIRT1 activity has been documented as beneficial in many disease models and human patients; therefore, it has been widely accepted that SIRT1-activating compounds could provide therapeutic benefits for various metabolic and degenerative diseases (Baur, 2010). On the other hand, the suppressing role for SIRT1 in p53 apoptotic activity suggests tumor-promoting properties of SIRT1 (Luo et al., 2001). Indeed SIRT1 protein has been reported to be elevated in

many types of neoplasia, including prostate cancer, acute myeloid leukemia, colon cancer, and various non-melanoma skin cancers (Deng, 2009). It has been recently reported that inhibiting SIRT1 activates p53, thus facilitating the elimination of leukemia stem cells (Li et al., 2012). Therefore, SIRT1-inhibiting compounds confer therapeutic potentials for various human malignancies. Resveratrol, a compound identified in a screen for SIRT1 activators, has been reported to increase lifespan in yeast, worms, and flies, and to enhance healthspan in rodents (Agarwal and Baur, 2011; Baur et al., 2006; Howitz et al., 2003; Milne et al., 2007; Wood et al., 2004). Beneficial effects of resveratrol have been reported on aging-related cataracts, reduced bone density, neurodegenerative diseases, obesity, and diabetes. Resveratrol induces multiple gene expression alterations, mimicking multiple gene expression alterations induced by calorie restriction (CR) (Pearson et al., 2008). Consumption of ResVida®, a resveratrol-containing composition, confers significant metabolic changes similar to that of CR in obese human individuals (Timmers et al., 2011). Studies involving another resveratrol-containing nutraceutical, Longevinex®, revealed that short-term consumption of the nutraceutical can recapitulate the long-term effects of CR (Barger et al., 2008).

A-type nuclear lamins, encoded by the *LMNA* locus, are type V intermediate filament proteins. The two most prominent A-type lamins, lamin A and C, only differ in the C-terminus where CaaX motif dictates a series of processing events including transient isoprenylation (Rusinol and Sinensky, 2006). A *de novo* G608G mutation in *LMNA* promotes alternate splicing, yielding a partially processed prelamin A (also referred to as progerin) that is the predominant cause of Hutchinson-Gilford Progeria Syndrome, a severe form of early-onset premature aging (Eriksson et al., 2003). Mice deficient for Zmpste24, a metalloproteinase responsible for prelamin A maturation, manifest many of the progeroid features resembling Hutchinson-Gilford progeria syndrome (HGPS) patients (Pendas et al., 2002).

The present inventors and other researchers have shown that HGPS skin fibroblasts and mouse embryonic fibroblasts (MEFs) derived from *Zmpste24*$^{-/-}$ embryos undergo early

senescence attributable to genomic instability and hyperactivation of the p53 pathway, and that reduction of the prelamin A level in *Zmpste24*$^{-/-}$ mice by *Lmna* heterozygosity ameliorates progeroid phenotypes and significantly extends lifespan (Fong et al., 2004; Liu et al., 2005; Varela et al., 2005). Human cells engineered to express progerin exhibited defective proliferation and premature senescence (Candelario et al., 2008; Kudlow et al., 2008).

Lamin A/C is a major component of the nuclear matrix (NM), a filamentous nucleoskeleton distinct from chromatin and important for maintaining nuclear structure (Fey et al., 1991). Chromatin and other proteins dynamically associate with the NM to regulate various nuclear activities, including replication, gene transcription, DNA repair, and chromatin organization (Blencowe et al., 1994; Kruhlak et al., 2000; Phair and Misteli, 2000). For example, the NM co-purifies with a majority of the nuclear histone deacetylase (HDAC) activity (Downes et al., 2000; Hendzel et al., 1991; Li et al., 1996). One of the hallmarks of HGPS and *Zmpste24*$^{-/-}$ cells is a misshaped nucleus, which leads to disorganized heterochromatin (Liu et al., 2005; Pendas et al., 2002; Scaffidi and Misteli, 2005) and mislocalized nuclear proteins, such as ATR, SKIP, XPA and Mof (Krishnan et al., 2011; Liu et al., 2005; Liu et al., 2008; Manju et al., 2006; Pendas et al., 2002; Scaffidi and Misteli, 2005, 2006, 2008). Rescue of nuclear shape abnormality by reducing unprocessed prelamin A or progerin from the nuclear envelope via treatment with farnesyl transferase inhibitor (FTI) significantly ameliorates progeroid features in both HGPS cells and mouse models (Capell et al., 2005; Fong et al., 2006; Glynn and Glover, 2005; Toth et al., 2005; Varela et al., 2008).

Alternate splicing events at the wild type *LMNA* locus can lead to expression of low levels of progerin, which may affect the normal aging process (Scaffidi and Misteli, 2006). An increased number of cells expressing progerin were found during aging in normal individuals (McClintock et al., 2007) and telomere shortening or dysfunction activates progerin production (Cao et al., 2011). These findings suggest that progerin may contribute to the normal aging

process (Burtner and Kennedy, 2010), possibly through modulating the activity of proteins implicated in aging.

Over the past several years, calorie restriction (CR)-mimicking properties of resveratrol and SIRT1 protein have attracted considerable efforts in searching for resveratrol mimics and SIRT1 activators. Compounds exhibiting significantly higher SIRT1-activating potential than resveratrol have been identified, and these compounds can elicit similar CR-mimicking beneficial effects as that of resveratrol. In addition, it has been reported that resveratrol specifically enhances SIRT1 activity towards a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys-Lys^{Ac}-AMC) (SEQ ID NO:1) rather than the unmodified one (Borra et al., 2005; Kaeberlein et al., 2005). This observation was later confirmed by other researchers, showing that resveratrol and SRT1720 do not confer any SIRT1 activation towards its full-length native target proteins, including p53 and PGC-1α (Beher et al., 2009; Dai et al., 2010; Pacholec et al., 2010). Therefore, despite various beneficial effects of resveratrol and mimics, the underlying mechanism is still unclear.

BRIEF SUMMARY OF THE INVENTION

In one embodiment, the present invention provides methods of modulating the deacetylase activity of SIRT1 in one or more cell by modifying the binding affinity of lamin A to SIRT1 via an interaction modifying compound. The deacetylase activity of SIRT1 can be increased by an increased binding affinity of lamin A to SIRT1 and decreased by a decreased binding affinity of lamin A to SIRT1. An example of an interaction modifying compound includes, but is not limited to, resveratrol. Resveratrol increases the binding affinity of lamin A to SIRT1 in embodiments described herein. In some embodiments of the present invention, SIRT1 deacetylase activity is modulated via an interaction modifying compound by enhancing the binding capacity of the carboxyl terminus of lamin A protein to SIRT1 protein.

In one embodiment, the present invention provides methods of screening for agents that modulate SIRT1 deacetylase activity based on the interaction between lamin A and SIRT1 proteins and SIRT1-activating/inhibiting properties of lamin A. Some methods include contacting a candidate molecule with cells expressing SIRT1 in a test sample; determining deacetylase activity in the test sample; and selecting the candidate molecule as an agent that modulates SIRT1 deacetylase activity if the molecule changes the level of SIRT1 deacetylase activity in the test sample. Candidate molecules that activate or enhance SIRT1 deacetylase activity include, but are not limited to, peptide fragments of lamin A, including peptide fragments of the carboxyl domain of lamin A; analogs of lamin A, including peptide fragments of such analogs; compounds that enhance the binding of lamin A to SIRT1; compounds that enhance or induce the expression of lamin A; and combinations thereof. Candidate molecules that inhibit or reduce SIRT1 deacetylase activity include, but are not limited to, peptide fragments of lamin A, including the carboxyl domain of the lamin A peptide; analogs of lamin A, including peptide fragments of such analogs; agents or compounds that inhibit lamin A activity; agents or compounds that inhibit expression of lamin A; and combinations thereof.

In one embodiment, the present invention provides a method for treating a disease or condition in which modulated SIRT1 deacetylase activity is beneficial. Methods include administering to a subject in need of such treatment, an effective amount of an agent that modulates SIRT1 deacetylase activity. In some embodiments, the agent administered increases SIRT1 deacetylase activity. Examples of SIRT1-activating compounds include, but are not limited to, peptide fragments of lamin A, including fragments of the carboxyl domain of lamin A; analogs of the lamin A, or fragments thereof; compounds that enhance the binding of lamin A to SIRT1; and combinations thereof. In other embodiments, the agent administered decreases SIRT1 deacetylase activity. Such agents include, but are not limited to, carboxyl terminal peptides of lamin A, and analogs of carboxyl terminal peptides of lamin A.

Embodiments of methods of the present invention that result in increased SIRT1 deacetylase activity are useful in treating diseases or conditions where it is beneficial to increase the number and/or function of bone marrow stromal cells and/or hematopoietic stem cells, such as, but not limited to, metabolic and/or aging-related degenerative diseases. As a result, for example, an increase in bone density and prevention of bone loss can occur. Furthermore, embodiments of methods of the present invention that result in decreased SIRT1 deacetylase activity are useful in treating neoplasia and other malignancies.

In one embodiment, the present invention provides methods for increasing SIRT1 deacetylase activity in one or more cell. Methods include administering to, or contacting, one or more cell that expresses SIRT1, and is in need of increased SIRT1 deacetylase activity, a lamin A peptide, an analog of the lamin A peptide, or functional fragment thereof, in an amount effective to increase the deacetylase activity of SIRT1. In one embodiment, the lamin A peptide useful according to the present invention is of human origin, having the amino acid sequence of (SEQ ID NO:2; GenBank Accession No. NP_733821), or an analog thereof. In one embodiment, the functional fragment of the lamin A peptide that increases SIRT1 deacetylase activity comprises the carboxyl domain of lamin A, or an analog thereof. The carboxyl domain of the lamin A peptide may include the amino acids 567-646 of SEQ ID NO:2, or one or more fragment thereof. Particularly useful fragments are from about 3 amino acids to about 50 amino acids in length.

In one embodiment, the present invention provides methods for decreasing or inhibiting SIRT1 deacetylase activity in one or more cell. Methods include administering to one or more cell that expresses SIRT1, and is in need of decreased SIRT1 deacetylase activity, an inhibitor of the lamin A protein or peptide. Lamin A inhibitors useful according to embodiments of the present invention include, but are not limited to, agents that inhibit lamin A activity; and agents that reduce or inhibit the expression of lamin A, such as agents that inhibit the transcription, translation, and/or processing of lamin A.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that SIRT1 interacts with lamin A, and that the formation of prelamin A/progerin jeopardizes the SIRT1-lamin A interaction. (**A**) FLAG-SIRT1 and lamin A were ectopically expressed in HEK293 cells. By Western blotting, lamin A was detected in anti-FLAG immunoprecipitates; FLAG-SIRT1 was detected in anti-lamin A/C immunoprecipitates. (**B**) In total cell lysate of HEK293 cells, endogenous SIRT1 was pulled down by anti-lamin A/C immunoprecipitates and reciprocally; endogenous lamin A was pulled down by anti-SIRT1 immunoprecipitates. (**C**) Immunofluorescence staining and confocal microscopy of SIRT1 and lamin A/C in human fibroblasts. Majority of nuclear SIRT1 co-localizes with lamin A in the nuclear interior (arrows). Scale bar, 5 μm. (**D**) Confocal microscopy showed co-localization of ectopic EGFP-SIRT1 and DsRed-lamin A in human fibroblast cells. Scale bar, 10 μm. (**E**) Recombinant human SIRT1 (rhSIRT1) was pulled down by anti-lamin A immunoprecipitates in test tubes containing rhSIRT1 and recombinant human lamin A (rhLamin A). (**F**) Lamin A, but not lamin C, was pulled down in anti-FLAG-SIRT1 immunoprecipitates in HEK293 cells. (**G**) HEK293 cells were transiently transfected with FLAG-SIRT1 together with one of the A-type lamins, *i.e.*, wild-type lamin A, unprocessible prelamin A, and progerin. Western blotting was performed to determine levels of A-type lamins in anti-FLAG immunoprecipitates. Note that significantly less prelamin A/progerin was pulled down by anti-FLAG antibody compared with wild-type lamin A. (**H**) Quantification of (**G**). Data represent mean ± SEM, n = 3. $^{**}P < 0.01$.

Figure 2 shows mislocalization and reduced deacetylase activity of SIRT1 in progeroid cells. (**A**) Representative immunoblot showing various proteins in nuclear (Nu, P1) and nuclear matrix (NM, P2') fractions. NM-associated SIRT1 was significantly reduced in *Zmpste24*$^{-/-}$ BMSCs, whereas levels of Sirt6, CBP, Foxo3a, histone H3 and β-actin were comparable between wild-type and *Zmpste24*$^{-/-}$ BMSCs in NM fraction. Total nuclear level of SIRT1 was not changed. (**B**) Quantification of (**A**). Data represent mean ± SEM, n = 3. $^{**}P < 0.01$. (**C**) Lamin A,

unprocessible prelamin A or progerin was stably expressed in HEK293 cells. Subcellular fractionation and Western blotting were performed to determine the NM associated SIRT1. While NM-association of SIRT1 was reduced in prelamin A- and progerin-transfected cells compared with wild-type lamin A, the levels of Foxo3a and β-Catenin remained unchanged. (**D**) Quantification of (**C**). Data represent mean ± SEM, n = 3. **P < 0.01. (**E**) Hyperacetylation of Foxo3a in *Zmpste24*$^{-/-}$ BMSCs determined by Western blotting with anti-acetyl lysine antibodies in anti-Foxo3a immunoprecipitates. (**F**) Quantification of (**E**). Data represent mean ± SEM, n = 3. *P < 0.05, **P < 0.01. (**G**) Upper, acetylation of Foxo3a in HEK293 cells expressing ectopic lamin A or prelamin A or progerin determined by Western blotting with anti-acetyl lysine antibodies in anti Foxo3a immunoprecipitates; lower, expression of catalase, MnSOD and GADD45α in the input.

Figure 3 shows that resveratrol directly activates SIRT1 in a lamin A-dependent manner. (**A**) RhSIRT1 deacetylase activity was determined by BioMol® SIRT1 Fluorimetric Drug Discovery Kit (BSDK) in the presence or absence of rhLamin A. Data represent mean ± SEM, n = 3. *P < 0.05, **P < 0.01, rhLamin A + rhSIRT1 Vs rhSIRT1 only. ***The molar ratio of rhLamin A to rhSIRT1 is 0.5, 1.0, 2.0, and 4.0 respectively. (**B**) Acetyl FLAG-p53 was incubated with rhSIRT1 and rhLamin A in the presence or absence of resveratrol. FLAG-p53 acetylation was detected by Western blotting with anti-acetyl lysine antibodies. Relative level of acetylated p53 was quantified by Image J®. *The molar ratio of rhLamin A to rhSIRT1 is 0.5, 1.0 and 2.0 respectively. (**C**) Level of rhSIRT1 pulled down by anti-lamin A/C antibody in the presence or absence of resveratrol was assessed by Western blotting. (**D**) FLAG-SIRT1 and lamin A were co-transfected into HEK293 cells. Cells were treated with resveratrol followed by anti-FLAG immunoprecipitation. Western blotting showed that resveratrol treatment increased the interaction between SIRT1 and lamin A in a dose-dependent manner. (**E**) Quantification of (**D**). Data represent mean ± SEM, n = 3. *P < 0.05. (**F**) Representative immunoblot in wild-type, SIRT1 null and *Lmna* null cells treated with different doses of resveratrol. (**G**)

Immunofluorescence staining of H3K9ac in wild-type and *Lmna* null cells treated or untreated with resveratrol. **(H)** Resveratrol enhanced the association of rhSIRT1 with NM in test tube. Recombinant hSIRT1 was incubated with NM fraction prepared from wild-type or *Zmpste24*$^{-/-}$ BMSCs in the presence and absence of resveratrol (10 μM) in a similar way as the SIRT1 deacetylase activity assay was performed. Insoluble NM and reaction buffer were separated by centrifugation. Western blotting and Coomassie Blue staining were performed to determine the level of rhSIRT1.

Figure 4 shows that resveratrol rescues the decline of bone marrow stromal cells (BMSCs) in *Zmpste24*$^{-/-}$ mice in SIRT1-dependent manner. **(A)** Resveratrol (10 μM) increased colony-forming capacity of *Zmpste24*$^{-/-}$ BMSCs. Colony-forming assay was performed on freshly isolated bone marrow cells in 10 cm dishes in the presence and absence of resveratrol. **(B)** Colony number counting. Data showed that resveratrol (RSV) increased colony-formation capacity of *Zmpste24*$^{-/-}$ BMSCs in a dose-dependent manner (2 μM and 10 μM). Data represent mean ± SEM, n = 4. **P* < 0.05, RSV Vs Vehicle. **(C)** Left, level of prelamin A pull down by anti SIRT1 immunoprecipitates in BMSCs treated or untreated with resveratrol (2 μM) was determined by Immunoblotting; right, level of acetylated Foxo3a in resveratrol was assessed by Immunoblotting. **(D)** Levels of catalase and Gadd45α in SIRT1 or scramble siRNA treated *Zmpste24*$^{-/-}$ BMSCs in the presence or absence of resveratrol (10 μM) determined by Western blotting. **(E)** Colony-forming capacity of *Zmpste24*$^{-/-}$ BMSCs by SIRT1 or scramble knocking down in the presence or absence of resveratrol. Resveratrol treatment (10 μM) increased the colony-forming capacity in *Zmpste24*$^{-/-}$ BMSCs (left) and this was completely abolished by knocking down SIRT1 (right). Colony forming assay was done on freshly isolated cells in 6-well plates. **(F)** Quantification of **(E)**. Data represent mean ± SEM, n = 5. **P* < 0.05, "Scramble + RSV" Vs "Scramble + Veh". **(G)** Levels of H3K9ac and IR-induced -H2AX with or without ectopic SIRT1 in wild-type and Zmpste24 null BMSCs were assessed by Western blotting. **(H)** Ectopic SIRT1 increased the colony-forming capacity of *Zmpste24*$^{-/-}$and wild-type BMSCs.

Colony-forming assay was performed on freshly isolated cells in 6 cm dishes. (**I**) Quantification of (**H**). Data represent mean ± SEM, n = 5. *$P < 0.05$, SIRT1 Vs Mock.

Figure 5 shows that resveratrol rescues ASC decline, ameliorates progeroid features, and extends lifespan in *Zmpste24*$^{-/-}$ mice. (**A**) Colony-forming capacity of BMSCs in *Zmpste24*$^{-/-}$ mice treated with either vehicle or resveratrol (20 μg/ml in drinking water). Colony-forming assays were performed on freshly isolated bone marrow cells in 10 cm dishes. (**B**) Quantification of (**A**). Data represent average colony number of BMSCs ± SEM, n=3. *P < 0.05. (**C**) Levels of catalase, acetylated p53 and H3K9ac in BMSCs isolated from vehicle-treated and resveratrol-treated *Zmpste24*$^{-/-}$ mice. (**D**) Feeding *Zmpste24*$^{-/-}$ mice with resveratrol increased HSC population. Each dot represents the percentage of HSC population in total bone marrow mononucleated cells in individual mouse. *$P < 0.05$, vehicle-treated *Zmpste24*$^{-/-}$ mice Vs wild-type and resveratrol-treated (20 μg/m1 in drinking water) Vs vehicle-treated *Zmpste24*$^{-/-}$ mice. (**E**) Micro-CT examination of trabecular bone structure in wild-type mice and in *Zmpste24*$^{-/-}$ mice treated with either resveratrol (20 μg/ml in drinking water) or vehicle. (**F**) Resveratrol-treatment increased bone mineral density in *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 3. *$P < 0.05$, vehicle-treated *Zmpste24*$^{-/-}$ mice Vs wild-type and resveratrol-treated (20 μg/ml in drinking water) Vs vehicle-treated *Zmpste24*$^{-/-}$ mice. (**G**) Body weight in resveratrol-treated and vehicle-treated male *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 10. *$P < 0.05$. (**H**) Body weight in resveratrol-treated and vehicle-treated female *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 10. *$P < 0.05$. (**I**) Survival rate in resveratrol-treated and vehicle-treated *Zmpste24*$^{-/-}$ mice. $P < 0.0001$. (**J**) Maximal lifespan in resveratrol-treated and vehicle-treated *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM. **$P < 0.01$.

Figure 6 shows that lamin A interacts with SIRT1. (**A**) Endogenous interaction between lamin A and SIRT1 in BMSCs was determined by co-immunoprecipitation. (**B**) Endogenous interaction between lamin A and SIRT1 in wilt-type and *Lmna* null mouse embryonic fibroblasts (MEFs) was determined by co-immunoprecipitation. (**C**) Interaction

between GFP-lamin A and SIRT1, SIRT2 and SIRT5 was assessed by anti GFP co-immunoprecipitation.

Figure 7 shows mislocalization and reduced deacetylase activity of SIRT1 in progeria cells and activation of SIRT1 by resveratrol in the presence of lamin A. (**A**) Expression of Kap-1 and Mcm3 in different subcellular fractions. Cells were fractionated and the subcellular distribution of Kap-1 and Mcm3 was determined by Western blotting. (**B**) *SIRT1*$^{-/-}$ and wild-type MEFs were fractionated into cytoplasmic (Cyto, S1), nucleoplasmic/chromatic (Np+Chr, S2'), and nuclear matrix (NM, P2') fractions. Representative immunoblot showing the NM association of SIRT1. (**C**) SIRT1 expression in nuclear and NM fractions determined by Western blotting in dermal fibroblasts derived from healthy individual and patients with different *LMNA* gene mutations. Note that the NM-associated SIRT1 was significantly down-regulated in HGPS cells compared to that in cells from healthy individual or from patients with non-progeria *LMNA* mutations. The total nuclear level of SIRT1 was also decreased in HGPS cells. (**D**) Quantification of SIRT1 in human cells. Significant down-regulation of NM-associated SIRT1 relative to total nuclear SIRT1 in HGPS fibroblasts, compared with that in dermal fibroblasts from non-progeria laminopathy patients and healthy individual. (**E**) RhSIRT1 deacetylase activity was determined in the presence of cytoplasmic or NM fraction. The relative increase in deacetylase activity after addition of rhSIRT1 to the assay buffer, cytoplasmic or NM was determined and plotted. The NM from wild-type BMSCs potentiated rhSIRT1 deacetylase activity whereas the stimulating capacity of NM from *Zmpste24*$^{-/-}$ BMSCs was greatly compromised. Data represent mean ± SEM, n = 3. $*P < 0.05$. (**F**) Acetyl FLAG-p53 was incubated with rhSIRT1 in the presence or absence of rhLamin A and resveratrol. FLAG-p53 acetylation was detected by Western blotting with anti-acetyl lysine antibodies. (**G**) Resveratrol enhanced NM-association of SIRT1 in wild-type and *Zmpste24*$^{-/-}$ BMSCs determined by Western blotting. (**H**) Quantification of (**G**). Data represent mean ± SEM, n = 3. $*P < 0.05$. (**I**) Nuclear matrix (NM) fraction preserves SIRT1 deacetylase activity. Recombinant human

SIRT1 (rhSIRT1) deacetylase activity was determined by BioMol® SIRT1 Fluorimetric Drug Discovery Kit in the presence or absence of nuclear matrix fraction. Suramin Sodium was applied as an inhibitor of SIRT1. The relative rhSIRT1 deacetylase activity was plotted. Data represent mean ± SEM, n = 3. *$P < 0.05$.

Figure 8 shows effects of resveratrol on *Zmpste24*$^{-/-}$ mouse embryonic fibroblasts (MEFs). (**A**) Cellular senescence determined by senescence-associated β-galactosidase assay in *Zmpste24*$^{-/-}$ MEFs treated with resveratrol. (**B**) Levels of p16^{ink4a} in resveratrol-treated MEF cells determined by Western blotting.

Figure 9 shows accelerated ASC decline in *Zmpste24*$^{-/-}$ mice**.** (**A**) Number of BMSC colonies after 12-days culture. Colony-forming assay was performed in 10 cm dishes with freshly isolated bone marrow cells from 4-month-old wild-type and *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 5. *$P < 0.01$. (**B**) Colonies of magnetically enriched BMSCs from either wild-type or mutant mice in 10 cm dishes after 12-days culture. (**C**) Proliferative capacity of enriched BMSCs from 1-month and 5-months wild-type and *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 3. **$P < 0.001$. (**D**) Senescence-associated β-galactosidase assay in enriched BMSCs from 1-month-old wild-type and *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n = 3. **$P < 0.001$. (**E**) Total number of mononucleated cells in femurs and tibias from 4-month-old wild-type and *Zmpste24*$^{-/-}$ mice. Data represent mean ± SEM, n =6. *$P < 0.05$. (**F**) Representative FACS profiles of HSCs in bone marrow from 4-month-old *Zmpste24*$^{-/-}$ and wild-type mice. Gate R4 represents HSC population (Lineage^{-}Flt3^{-}Sca-1^{+}c-Kit^{high}). Note reduced HSC population in *Zmpste24*$^{-/-}$ mice. (**G**) Percentage of HSC population in total mononucleated cells in bone marrow of 1-month-old, 2-month-old and 4-month-old wild-type and *Zmpste24*$^{-/-}$mice. *$P < 0.01$. (**H**) HSCs from 1-month-old wild-type or *Zmpste24*$^{-/-}$ mice were transplanted into lethally irradiated recipients. In wild-type transplanted recipients, B cell differentiation was not affected at 1, 4 or 6 months; in recipients repopulated with Zmpste24$^{-/-}$

donors, the B cell lineage was greatly reduced 4 months after transplantation. $^{**}P < 0.001$, *Zmpste24*$^{-/-}$ vs *Zmpste24*$^{+/+}$.

Figure 10 shows that N-acetyl cysteine feeding rescues ASC decline and extends lifespan in *Zmpste24*$^{-/-}$mice. (**A**) Colony forming unit-fibroblast (CFU-F) of enriched BMSCs determined in the presence or absence of H_2O_2. Data represent mean ± SEM, n = 3. $^{*}P < 0.05$. (**B**) Elevated ROS level in freshly isolated *Zmpste24*$^{-/-}$ HSCs compared with that in wild-type HSCs (left) and rescued ROS level by N-acetyl Cysteine (NAC, 1 mg/ml in drinking water) (right). (**C**) Colony-forming efficiency of BMSCs in *Zmpste24*$^{-/-}$ mice treated with either vehicle or NAC (1 mg/ml in drinking water). Colony-forming assays were performed on freshly isolated bone marrow cells in 10 cm dishes. Data represent mean ± SEM, n = 3. $^{*}P < 0.05$. (**D**) Percentage of bone marrow HSC population in vehicle-treated and NAC-treated *Zmpste24*$^{-/-}$ mice. $^{*}P < 0.05$, vehicle-treated *Zmpste24*$^{-/-}$ mice Vs wild-type and NAC-treated Vs vehicle-treated *Zmpste24*$^{-/-}$ mice. (**E**) Body weight of vehicle-treated and NAC-treated *Zmpste24*$^{-/-}$ mice at 4-months of age. Data represent mean ± SEM, n =12. $^{*}P < 0.05$, NAC-treated Vs vehicle-treated. (**F**) Survival rate of vehicle-treated and NAC-treated *Zmpste24*$^{-/-}$ mice. $P < 0.0001$.

Figure 11 (**A**) Recombinant human SIRT1 (rhSIRT1) was pulled down by anti lamin A immunoprecipitates in test tube containing rhSIRT1 and recombinant human lamin A (rhLamin A). (**B**) RhSIRT1 deacetylase activity was determined by BioMol® SIRT1 Fluorimetric Drug Discovery Kit (BSDK) in the presence or absence of rhLamin A. Data represent mean ± SEM, n = 3. $^{*}P < 0.05$, $^{**}P < 0.01$, rhLamin A + rhSIRT1 Vs rhSIRT1 only. ***The molar ratio of rhLamin A to rhSIRT1 is 0.5, 1.0, 2.0, and 4.0 respectively. (**C**) Acetyl FLAG-p53 was incubated with rhSIRT1 in the presence or absence of rhLamin A. FLAG-p53 acetylation was detected by Western blotting with anti-acetyl lysine antibodies. Relative level of acetylated p53 was quantified by Image J®. *The molar ratio of rhLamin A to rhSIRT1 is 0.5 and 1.0 respectively. (**D**) RhSIRT1 deacetylase activity was determined by BioMol® SIRT1

Fluorimetric Drug Discovery Kit (BSDK) in the presence or absence of LA-80 (synthetic peptide of carboxyl 80 aa of lamin A). Data represent mean ± SEM, n = 3. $^{**}P < 0.01$, LA-80 + rhSIRT1 Vs rhSIRT1 only. (**E**) Acetyl FLAG-p53 was incubated with rhSIRT1 in the presence of various amount of LA-80. FLAG-p53 acetylation was detected by Western blotting with anti-acetyl lysine antibodies (left). Relative level of acetylated p53 was quantified by Image J® (right).

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is the amino acid sequence useful according to the present invention.

SEQ ID NO:2 is the amino acid sequence of the human lamin A protein.

DETAILED DISCLOSURE OF THE INVENTION

In one embodiment, the present invention provides methods of screening SIRT1-activating/inhibiting compounds based on the interaction between lamin A and SIRT1 proteins and SIRT1-activating property of lamin A. In another embodiment, the present invention provides uses of SIRT1-activating compounds to treat patient(s)/subject(s) suffering from metabolic and/or aging-related degenerative diseases, and uses of SIRT1-inhibiting compounds to treat human malignancies. In another embodiment, the present invention provides methods of modulating the deacetylase activity of SIRT1 by modifying the binding affinity of lamin A to SIRT1 via interaction modifying compound(s).

Activation of SIRT1 Deacetylase Activity by Lamin A

Given the essential roles of the nuclear matrix (NM) in preserving HDAC activity and the longevity-promoting properties of resveratrol, the potential effect of lamin A on SIRT1 is determined. The results show that lamin A directly interacts with SIRT1 and serves as an activator of SIRT1 on the NM; prelamin A and progerin exhibit significantly reduced binding

capacity to SIRT1, therefore mis-localize SIRT1 from the NM, leading to rapid decline of ASCs in *Zmpste24*$^{-/-}$ mice. Resveratrol increases the binding of SIRT1 with A-type lamins both *in vitro* and *in vivo,* and thus can enhance the deacetylase activity of SIRT1, restore ASC population, ameliorate progeroid features, and extend lifespan in *Zmpste24*$^{-/-}$ mice.

The present invention shows that lamin A is an activator of SIRT1; Resveratrol activates SIRT1 via increasing its interaction with lamin A; Resveratrol rescues ASC decline in the SIRT1-dependent manner; and Resveratrol-treatment alleviates progeroid features and extends lifespan in progeria mice.

The present invention shows that nuclear lamin A protein forms complex with longevity/anti-aging SIRT1 protein *in vivo*. Lamin A protein directly binds to SIRT1 protein. Lamin A activates SIRT1 deacetylase activity towards both a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys- Lys^{Ac}-AMC) and a full-length acetylated FLAG-p53 protein (a known target of SIRT1 protein). Synthesized peptide, containing 80 amino acids on the carboxyl terminus of lamin A protein, confers much higher activating potential on SIRT1 towards both a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys- Lys^{Ac}-AMC) and a full-length acetylated FLAG-p53 protein.

In one embodiment, the present invention provides methods of screening small peptides of lamin A protein that activate SIRT1 deacetylase activity. In another embodiment, the present invention provides methods for identifying lamin A-peptide-mimicking compounds to activate or inhibit SIRT1 protein. In one embodiment, the present invention provides methods for identifying compounds that modulate the interaction between lamin A protein and SIRT1 protein thus to activate or inhibit the deacetylase activity of SIRT1. In a further embodiment, the present invention provides methods for treatment of patients suffering various metabolic diseases, such as obesity, cardiovascular diseases, diabetes, neurodegenerative diseases, premature aging syndromes and aging. Also provided are methods of using SIRT1-inhibiting compounds for the

treatment of human cancers, including prostate cancer, acute myeloid leukemia, colon cancer and various non-melanoma skin cancers.

The present inventors have discovered that the nuclear lamin A protein interacts with the longevity-promoting/anti-aging SIRT1 protein. In one embodiment, the present invention provides methods of screening SIRT1 activators or inhibitors. In another embodiment, the present invention provides methods of increasing or inhibiting SIRT1 activity to treat human metabolic and degenerative diseases as well as neoplasia.

In one embodiment, the invention provides a method of using small carboxyl terminal peptides of lamin A protein to enhance SIRT1 protein activity. Synthesized peptides ranging from 3 mer to 20 mer located in the G_{567}-Y_{646} region of mature lamin A protein are determined for their ability to enhance SIRT1 deacetylase activity towards both a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys- Lys^{Ac}-AMC) and a full-length acetylated FLAG-p53 protein (a known target of SIRT1 protein) *in vitro*. In a further embodiment, the effects of synthesized 3 mers to 20 mers peptides of SIRT1 are tested in cells using the acetylation status of SIRT1 direct deacetylating targets, such as p53 and PGC-1α, as readout. In a still further embodiment, covalently modified aforementioned 3 mer to 20 mer lamin A peptides are tested in the ability to enhance SIRT1 deacetylase activity by aforementioned *in vitro* and *in vivo* assays.

In one embodiment, the present invention provides a method of screening compounds that mimic the structure of aforementioned 3 mer to 20 mer lamin A peptides, wherein enhanced deacetylase activity of SIRT1 is determined by a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys- Lys^{Ac}-AMC) and a full-length acetylated FLAG-p53 protein in the test tube. In a further embodiment, the effects of lamin A-peptide-mimics on SIRT1 are tested in cells using the acetylation status of SIRT1 direct deacetylating targets, such as p53 and PGC-1 α, as readout.

In one embodiment, the present invention provides a method of identifying compounds capable of enhancing SIRT1 deacetylase activity via increasing the interaction between lamin A

and SIRT1 proteins. SIRT1 deacetylase towards both a fluorophore-conjugated synthetic peptide (Ac-Arg-His-Lys- Lys^{Ac}-AMC) and a full-length acetylated FLAG-p53 protein is determined in the presence of lamin A protein and tested compound. The ability of tested compounds to enhance the interaction between lamin A and SIRT1 in the test tube containing recombinant lamin A and recombinant SIRT1 and in cells can be determined by co-immunoprecipitation, Western blotting, and GST-pull down assays. The ability of tested compounds to activate SIRT1 can be tested in cells by examining the level of acetylated p53 and acetylated PGC-1α.

In one embodiment, this invention provides a method of using aforementioned SIRT1-activating formula, including small lamin A peptides, peptide-mimics and lamin A-SIRT1 interaction enhancing compounds, to treat human metabolic and aging-related degenerative diseases. In one embodiment, the candidate compounds/peptides can be tested in animal (such as mouse) models recapitulating metabolic and aging-related degenerative diseases, such as mice fed with high-fat diet, mouse models resembling Hutchinson-Gilford progeria syndrome (HGPS), db/db diabetic mice, etc.

In another embodiment, this invention provides a method of using SIRT1-inhibiting compounds, including small lamin A peptides, peptide-mimics, and lamin A-SIRT1 interaction inhibiting compounds, to treat human malignancies. In addition, the present invention provides methods for treating metabolic diseases, including obesity, diabetes, neurodegenerative diseases, cardiovascular diseases, premature aging syndromes, and aging, via the administration of SIRT1-activating compounds.

Lamin A directly interacts with SIRT1, and the last 80 amino acids on the carboxyl domain of lamin A serves as an activator of SIRT1. In one embodiment, the present invention provides a method for increasing SIRT1 deacetylase activity, wherein the method comprises administering to a cell that expresses SIRT1 and is in need of increased SIRT1 deacetylase activity a lamin A peptide, an analog of the lamin A peptide, or functional fragment thereof.

Amino acid sequences of various species of the Lamin A protein are publicly known, and can be readily obtained by a person skilled in the art, such as via the GenBank database. In one embodiment, the lamin A peptide useful according to the present invention is of human origin, having the amino acid sequence of SEQ ID NO:2; GenBank Accession No. NP_733821.

In one embodiment, the functional fragment of the lamin A peptide that increases SIRT1 deacetylase activity comprises the carboxyl domain of the lamin A peptide. In one embodiment, the functional fragment of the lamin A peptide that increases SIRT1 deacetylase activity comprises amino acids 570-664 of SEQ ID NO:2.

Inhibition of SIRT1 Deacetylase Activity

In one embodiment, the present invention provides a method for decreasing or inhibiting SIRT1 deacetylase activity, wherein the method comprises administering to a cell that expresses SIRT1, and is in need of decreased SIRT1 deacetylase activity, an inhibitor of the lamin A protein or peptide.

Lamin A inhibitors useful according to the present invention include, but are not limited to, agents that inhibit lamin A activity; and agents that reduce or inhibit the expression of lamin A, such as agents that inhibit the transcription, translation, and/or processing of lamin A.

Agents that inhibit lamin A activity include, but are not limited to, anti- lamin A antibodies, aptamers, lamin A binding partners, and small molecule inhibitors of lamin A.

In one embodiment, the lamin A inhibitor is an antibody, aptamer, or binding partner that binds to lamin A. In a specific embodiment, the lamin A inhibitor is an antibody, aptamer, or binding partner that binds specifically to lamin A. In a further specific embodiment, the lamin A inhibitor is an antibody, aptamer, or binding partner that binds specifically to human lamin A. In a further specific embodiment, the lamin A inhibitor is an antibody, aptamer, or binding partner that binds specifically to a human lamin A of SEQ ID NO:2.

In certain embodiments, the lamin A inhibitor is an antibody, aptamer, or binding partner that binds specifically to a lamin A protein of non-human animal species including, but not limited to, apes, chimpanzees, orangutans, monkeys, dogs, cats, horses, pigs, sheep, goats, chickens, mice, rats, and guinea pigs. Antibodies that bind specifically to lamin A proteins are commercially available. The skilled artisan can readily make antibodies, aptamers, or binding partners that specifically bind to lamin A proteins that are publically known. In another embodiment, the lamin A inhibitor is a fusion construct comprising the antibody, aptamer, or binding partner that binds specifically to a lamin A protein (such as human lamin A).

"Specific binding" or "specificity" refers to the ability of a protein to detectably bind an epitope presented on a protein or polypeptide molecule of interest, while having relatively little detectable reactivity with other proteins or structures. Specificity can be relatively determined by binding or competitive binding assays, using, *e.g.*, Biacore instruments. Specificity can be exhibited by, *e.g.*, an about 10:1, about 20:1, about 50:1, about 100:1, 10.000:1 or greater ratio of affinity/avidity in binding to the specific target molecule versus nonspecific binding to other irrelevant molecules.

Anti-lamin A antibodies of the present invention can be in any of a variety of forms, including intact immunoglobulin molecules, fragments of immunoglobulin molecules such as Fv, Fab and similar fragments; multimers of immunoglobulin molecules (*e.g.*, diabodies, triabodies, and bi-specific and tri-specific antibodies, as are known in the art; *see, e.g.*, Hudson and Kortt, J. Immunol. Methods 231:177 189, 1999); fusion constructs containing an antibody or antibody fragment; and human or humanized immunoglobulin molecules or fragments thereof.

Antibodies within the scope of the invention can be of any isotype, including IgG, IgA, IgE, IgD, and IgM. IgG isotype antibodies can be further subdivided into IgG1, IgG2, IgG3, and IgG4 subtypes. IgA antibodies can be further subdivided into IgA1 and IgA2 subtypes.

Antibodies of the present invention include polyclonal and monoclonal antibodies. The term "monoclonal antibody," as used herein, refers to an antibody or antibody fragment obtained

from a substantially homogeneous population of antibodies or antibody fragments (*i.e.* the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules).

A monoclonal antibody composition is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) only one type of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. Such antibodies were first described by Kohler and Milstein, *Nature*, 1975, 256:495-497, the disclosure of which is herein incorporated by reference. An exemplary hybridoma technology is described by Niman et *al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 1983, 80:4949-4953. Other methods of producing monoclonal antibodies, a hybridoma cell, or a hybridoma cell culture are also well known. See *e.g.*, Antibodies: A Laboratory Manual, Harlow *et al.*, Cold Spring Harbor Laboratory, 1988; or the method of isolating monoclonal antibodies from an immunological repertoise as described by Sasatry, *et al.*, *Proc. Natl. Acad. Sci. USA*, 1989, 86:5728-5732; and Huse *et al.*, *Science*, 1981, 246:1275-1281. The references cited are hereby incorporated herein by reference.

In some embodiments, lamin A inhibitors useful according to the present invention are agents that reduce or inhibit the expression of lamin A, such as agents that inhibit the transcription, translation, and/or processing of lamin A.

In an embodiment, the lamin A inhibitor is a lamin A antisense polynucleotide. In an embodiment, the lamin A inhibitor is an antisense polynucleotide that targets human lamin A mRNA. In some embodiments, the lamin A antisense polynucleotides target lamin A mRNAs of non-human animals including, but not limited to, apes, chimpanzees, orangutans, monkeys, dogs, cats, horses, pigs, sheep, goats, chickens, mice, rats, and guinea pigs. The skilled artisan would readily appreciate that the antisense polynucleotides can be designed to target any lamin A mRNAs publically known.

In some embodiments, the lamin A inhibitor is a siRNA having a sequence sufficiently complementary to a target lamin A mRNA sequence to direct target-specific RNA interference (RNAi). In some embodiments, the lamin A inhibitor is siRNA having a sequence sufficiently complementary to a target human lamin A mRNA sequence to direct target-specific RNA interference.

Examples of antisense polynucleotides include, but are not limited to, single-stranded DNAs and RNAs that bind to complementary target lamin A mRNA and inhibit translation and/or induce RNaseH-mediated degradation of the target transcript; siRNA oligonucleotides that target or mediate lamin A mRNA degradation; ribozymes that cleave lamin A mRNA transcripts; and nucleic acid aptamers and decoys, which are non-naturally occurring oligonucleotides that bind to and block lamin A protein targets in a manner analogous to small molecule drugs.

The term "nucleotide" refers to a nucleoside having one or more phosphate groups joined in ester linkages to the sugar moiety. Exemplary nucleotides include nucleoside monophosphates, diphosphates and triphosphates. The terms "polynucleotide" and "nucleic acid molecule" are used interchangeably herein and refer to a polymer of nucleotides joined together by a phosphodiester linkage between 5' and 3' carbon atoms. The terms "nucleic acid" or "nucleic acid sequence" encompass an oligonucleotide, nucleotide, polynucleotide, or a fragment of any of these, DNA or RNA of genomic or synthetic origin, which may be single-stranded or double-stranded and may represent a sense or antisense strand, peptide nucleic acid (PNA), or any DNA-like or RNA-like material, natural or synthetic in origin. As will be understood by those of skill in the art, when the nucleic acid is RNA, the deoxynucleotides A, G, C, and T are replaced by ribonucleotides A, G, C, and U, respectively.

As used herein, the term "RNA" or "RNA molecule" or "ribonucleic acid molecule" refers generally to a polymer of ribonucleotides. The term "DNA" or "DNA molecule" or deoxyribonucleic acid molecule" refers generally to a polymer of deoxyribonucleotides. DNA

and RNA molecules can be synthesized naturally *(e.g.*, by DNA replication or transcription of DNA, respectively). RNA molecules can be post-transcriptionally modified. DNA and RNA molecules can also be chemically synthesized. DNA and RNA molecules can be single-stranded (*i.e.*, ssRNA and ssDNA, respectively) or multi-stranded (*e.g.*, double stranded, *i.e.*, dsRNA and dsDNA, respectively). Based on the nature of the invention, however, the term "RNA" or "RNA molecule" or "ribonucleic acid molecule" can also refer to a polymer comprising primarily (*i.e.*, greater than 80% or, preferably greater than 90%) ribonucleotides but optionally including at least one non-ribonucleotide molecule, for example, at least one deoxyribonucleotide and/or at least one nucleotide analog.

As used herein, the term "nucleotide analog", also referred to herein as an "altered nucleotide" or "modified nucleotide" refers to a non-standard nucleotide, including non-naturally occurring ribonucleotides or deoxyribonucleotides. Preferred nucleotide analogs are modified at any position so as to alter certain chemical properties of the nucleotide yet retain the ability of the nucleotide analog to perform its intended function.

As used herein, the term "RNA interference" ("RNAi") refers to a selective intracellular degradation of RNA. RNAi occurs in cells naturally to remove foreign RNAs (*e.g.*, viral RNAs). Natural RNAi proceeds via fragments cleaved from free dsRNA which direct the degradative mechanism to other similar RNA sequences. Alternatively, RNAi can be initiated by the hand of man, for example, to silence the expression of endogenous target genes, such as lamin A.

As used herein, the term "small interfering RNA" ("siRNA") (also referred to in the art as "short interfering RNAs") refers to an RNA (or RNA analog) comprising between about 10-50 nucleotides (or nucleotide analogs) which is capable of directing or mediating RNA interference.

As used herein, a siRNA having a "sequence sufficiently complementary to a target mRNA sequence to direct target-specific RNA interference (RNAi)" means that the siRNA has a sequence sufficient to trigger the destruction of the target mRNA (*e.g.*, lamin A mRNA) by the

RNAi machinery or process. "mRNA" or "messenger RNA" or "transcript" is single-stranded RNA that specifies the amino acid sequence of one or more polypeptides. This information is translated during protein synthesis when ribosomes bind to the mRNA.

The present invention also contemplates vectors (*e.g.*, viral vectors) and expression constructs comprising the nucleic acid molecules useful for inhibiting lamin A expression and/or activity. In an embodiment, the vector comprises a siRNA that targets lamin A mRNA. In another embodiment, the vector comprises a nucleic acid molecule encoding an anti-lamin A antibody.

As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described, wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

Expression constructs of the invention will also generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in, for example, bacterial host cells, yeast host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence encoding a peptide of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. In a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site as it is from the transcription start site in its natural genetic environment.

Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

Drug Screening Assays

In one embodiment, the present invention pertains to methods for screening for therapeutic agents that increase or decrease SIRT1 deacetylase activity. The therapeutic agent can be a drug, chemical, compound, protein or peptide, or a nucleic acid molecule (*e.g.* DNA, RNA such as siRNA).

In one embodiment, the present invention provides a method for screening for agents that increase SIRT1 deacetylase activity, including contacting a candidate molecule with cells expressing SIRT1 in a test sample; determining deacetylase activity in the test sample; and selecting the candidate molecule as the agent that increases SIRT1 deacetylase activity if said molecule increases the level of SIRT1 deacetylase activity in the test sample.

In one embodiment of a screening assay for agents that increase SIRT1 deacetylase activity, the candidate molecule is selected from a fragment of lamin A peptide (such as a fragment of lamin A peptide of SEQ ID NO:2); an analog of compound mimicking lamin A activity, such as a peptidomimetic; or a compound enhancing lamin A-SIRT1 interaction.

Functional fragments of lamin A peptide can be of 5-600 amino acids in length, or of any length therebetween, including, but not limited to, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 , 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, and 600 amino acids in length.

In another embodiment, the screening assay for agents that increase SIRT1 deacetylase activity further includes determining binding of the candidate molecule to SIRT1, and selecting the candidate molecule if said molecule binds to SIRT1 or enhances the binding of lamin A (or a functional fragment thereof) to SIRT1.

In one embodiment, the present invention provides a method for screening for agents that decrease or inhibit SIRT1 deacetylase activity, including contacting a candidate molecule with

cells expressing SIRT1 in a test sample; determining deacetylase activity in the test sample; and selecting the candidate molecule as the agent that decreases or inhibits SIRT1 deacetylase activity if said molecule decreases or inhibits the level of SIRT1 deacetylase activity in the test sample.

In one embodiment of a screening assay for agents that decrease or inhibit SIRT1 deacetylase activity, the candidate molecule is selected from agents that inhibit lamin A activity; and agents that reduce or inhibit the expression of lamin A, such as agents that inhibit the transcription, translation, and/or processing of lamin A.

In one embodiment, the screening assay for agents that decrease or inhibit SIRT1 deacetylase activity further includes determining binding of the candidate molecule to SIRT1, and selecting the candidate molecule if said molecule inhibits the binding of lamin A to SIRT1.

In certain embodiments, the screening assays examine the i*n vitro* activity of SIRT1 in deacetylating proteins selected from KU70, Nbs1, p53, NF-κB, PPARγ, PGC-1α, FOXO, and SUV39H1. In certain embodiments, the screening assays examine the i*n vitro* activity of SIRT1 in deacetylating proteins selected from Ac-Arg-His-Lys- Lys^{Ac}-AMC (SEQ ID NO:1) or a full-length acetylated FLAG-p53 protein.

The deacetylation activity can be determined by methods including, but not limited to, co-immunoprecipitation, Western blotting, ELISA, immunofluorescence, radioimmunoassay, immunocytochemistry, and a combination thereof.

In certain embodiments, the present invention provides methods including determining SIRT1 activating peptide or mimics on the kinetics (V_{max} and K_m) of SIRT1 recombinant protein; determining the structure of SIRT1 in the presence or absence of peptide activator; and determining the biological effects of the peptide activator(s) on cultured human cells and premature aging mouse models.

Treatment of Diseases

In one embodiment, the present invention provides a method for treating a disease or condition in which increased SIRT1 deacetylase activity is beneficial, including administering to a patient or subject in need of such treatment an effective amount of lamin A peptide, an analog of lamin A that increases SIRT1 deacetylase activity or a functional fragment thereof.

In various embodiments, diseases or conditions in which increased SIRT1 deacetylase activity would be beneficial and which can be treated in accordance with the present invention include, but are not limited to, metabolic diseases, such as obesity, diabetes; neurodegenerative diseases, such as Alzheimer's Diseases; and aging-related diseases.

In one embodiment, the present invention provides a method for treating a disease or condition in which decreased SIRT1 deacetylase activity is beneficial, including administering to a patient or subject in need of such treatment, an effective amount of inhibitors of the lamin A peptide. In one embodiment, diseases or conditions in which decreased SIRT1 deacetylase activity would be beneficial include, but are not limited to, neoplasia.

The term "subject" or "patient," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the present invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals such as dogs, and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters.

The term "treatment" or any grammatical variation thereof (*e.g.*, treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, alleviating a symptom of a disease or condition; and/or reducing, suppressing, inhibiting, lessening, or affecting the progression, severity, and/or scope of a disease or condition.

The term “effective amount,” as used herein, refers to an amount that is capable of treating, preventing, or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect.

Therapeutic Compositions and Formulations

The present invention also provides for therapeutic or pharmaceutical compositions including a compound of the invention in a form that can be combined with a pharmaceutically acceptable carrier. In this context, the compound may be, for example, isolated or substantially pure.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Particularly preferred pharmaceutical carriers for treatment of or amelioration of inflammation in the central nervous system are carriers that can penetrate the blood/brain barrier. As used herein carriers do not include the natural plant material as it exists in nature.

Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The therapeutic composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, capsules, powders, sustained-release formulations and the like. The composition can be formulated with traditional binders and carriers such as triglycerides. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions contain a therapeutically effective amount of the therapeutic composition,

together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In one embodiment, the present invention provides pharmaceutical compositions adapted for local injection administration to human beings. Typically, compositions for local injection administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The therapeutic or pharmaceutical compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, *e.g.*, compound, carrier, of the pharmaceutical compositions of the invention.

Routes of Administration

The compounds and compositions of the subject invention can be administered to the subject being treated by standard routes, including oral, inhalation, or parenteral administration including intravenous, subcutaneous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intracardiac, transtracheal,

subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection, infusion, and electroporation, as well as co-administration as a component of any medical device or object to be inserted (temporarily or permanently) into a subject.

The amount of the therapeutic or pharmaceutical composition of the invention which is effective in the treatment of a particular disease, condition or disorder will depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. In general, the dosage ranges from about 0.001 mg/kg to about 3 g/kg. For instance, suitable unit dosages may be between about 0.01 to about 3 g, about 0.01 to about 1 g, about 0.01 to about 500 mg, about 0.01 to about 400 mg, about 0.01 to about 300 mg, about 0.01 to about 200 mg, about 0.01 to about 100 mg, about 0.01 to about 50 mg, about 0.01 to about 30 mg, about 0.01 to about 20 mg, about 0.01 to about 10 mg, about 0.01 to about 5 mg, about 0.01 to about 3 mg about, 0.01 to about 1 mg, or about 0.01 to about 0.5 mg. Such a unit dose may be administered more than once a day, e.g. two or three times a day.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary, depending on the type of the condition and the subject to be treated. In general, a therapeutic composition contains from about 5% to about 95% active ingredient (w/w). More specifically, a therapeutic composition contains from about 20% (w/w) to about 80% or about 30% to about 70% active ingredient (w/w).

Once improvement of the patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced as a function of the symptoms to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may however require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, condition or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

MATERIALS AND METHODS

Cell lines, constructs and antibodies

HEK293 cells, MEFs, and human dermal fibroblasts were maintained in DMEM supplemented with 10% FBS. HGADFN143, HGADFN188, HGADFN164, and HGADFN122 skin fibroblasts derived from HGPS patients were provided by Progeria Research Foundation. Human healthy dermal fibroblasts PH and cells harboring *LMNA* mutations, i.e. R453W, R482W and R401C, were provided by Professor Manfred Wehnert (Institute of Human Genetics, University of Greifswald, Greifswald, Germany). SIRT1 null MEFs were provided by Professor Chu-Xia Deng (NIDDK, National Institutes of Health, USA). F2-S human fibroblasts and preparation of MEFs from mouse embryos were described elsewhere (Liu et al., 2005).

Transfection was performed with Lipofectamine2000® (Invitrogen, USA) according to the manufacture's procedures. SIRT1 siRNA oligos were purchased from Invitrogen, USA. Lamin A and unprocessible prelamin A constructs have been described previously (Liu et al., 2005). The progerin construct was generated by bacterial recombineering based on the lamin A construct. Flag-FOXO3A (Addgene plasmid 8360) was obtained from Dr M. E Greenberg (Brunet et al., 2004). Adenoviral SIRT1 construct (Addgene plasmid 8438) (Rodgers et al., 2005) was provided by Dr P Puigserver. EGFP-SIRT1 construct (Sun et al., 2007) was provided by Prof Qiwei Zhai (Shanghai Institutes for Biological Sciences, China). Flag-tagged

SIRT1 mutants, Flag-p53, and p300 constructs were gifts from Dr. Zhenkun Lou (Mayo Clinic College of Medicine, USA).

Rabbit anti-SIRT6, anti-SIRT1, anti-CBP, and anti-acetyl lysine antibodies were obtained from Abcam (Cambridge, UK). Rabbit anti-H3K9ac and mouse anti-γ-H2AX antibodies were purchased from Millipore (Bedford, MA, USA). Anti-SIRT1, anti-lamin A/C, anti-catalase, anti-MnSOD, and anti-Gadd45α antibodies were purchased from Santa Cruz (Santa Cruz, CA, USA). Rabbit anti-Foxo3a was purchased from BioVision (Mountain View, CA, USA). Biotin-labeled lineage markers were purchased from BD Biosciences (San Jose, CA, USA). PE anti-mouse CD105 antibody was purchased from eBioscience (San Diego, CA, USA).

Resveratrol treatment of Zmpste24$^{-/-}$ mice

Zmpste24$^{-/-}$ mice have been described previously (Pendas et al., 2002). Mouse experimentation was performed in accordance with the guidelines of the Committee on the Use of Live Animals in Teaching and Research (CULATR) at the University of Hong Kong. New-born *Zmpste24$^{-/-}$* mice and wild-type controls were fed with resveratrol (20 μg/ml) or N-acetyl Cysteine (1 mg/ml) in drinking water. The survival of resveratrol-treated, NAC-treated, or vehicle-treated *Zmpste24$^{-/-}$* mice was recorded and their body weight was monitored weekly. The trabecular bone organization and bone mineral density were determined by micro-CT. The survival rate was analyzed by Kaplan-Meier method and statistical comparison was performed by Log-rank (Mantel-Cox) Test.

Bone marrow stromal cells and hematopoietic stem cells

Bone marrow stromal cells were isolated and cultured according to modified protocol (Enumeration and Expansion of Mouse Mesenchymal Progenitor Cells Using MesenCult®, Stemcell Technologies, Canada). Briefly, bone marrow cells were flushed out from femurs and tibias with α-MEM (Invitrogen, Carlsbad, CA, USA) supplemented with 20% FBS, and plated in

petri dishes. Non-adherent cells were removed on day 3 and medium was replaced every 3 days. BMSC colonies were fixed with methanol and stained with crystal violet solution on the indicated day. For magnetic enrichment, CD11b-positive population was removed using a MidiMACS™ magnetic cell sorting kit (Miltenyi Biotec, Bergisch Gladbach, Germany), following the manufacturer's instructions. Colonies containing more than 50 cells were counted. BMSC colony forming efficiency was calculated as number of colonies formed by 10^7 bone marrow nucleated cells.

To analyze hematopoietic stem cells, mononucleated cells were collected by flushing femurs and tibias with staining medium (HBSS supplemented with 2% FBS) and stained with biotin-coupled lineage markers, Biotin-Flt3, PE-Sca-1, APC-c-Kit, and then SAv-PerCP. FACS profile analyses were performed using a BD FACSCalibur.

To purify HSCs, red blood cells were lysed using NH_4Cl before surface-marker staining, and HSCs were then sorted with a BD FACSVantage SE. Sorted HSCs were incubated with 5 μM DCF-DA at 37°C for 30 min and analyzed by FACS at 488-nm excitation and 525-nm emission to determine the ROS level.

For hematopoietic reconstitution, recipient mice (B6SJL/BoyJ) were lethally irradiated with a dose of 9 Gy using a Gammacell 3000 Elan irradiator and 500 purified donor HSCs were injected into recipients via the tail vein. After repopulation, peripheral blood was collected, stained for cell surface markers, and analyzed by FACS.

SA-β-galactosidase assay

SA-β-galactosidase assay was performed using a Cellular Senescence Assay Kit (Chemicon International, CA, USA), following the manufacturer's instructions. For MEFs, resveratrol was supplemented in the complete medium at passage 4 and SA-β-galactosidase assay was performed at passage 6. To quantify SA-β-galactosidase staining, the blue-dyed precipitate was extracted with 100 μl DMSO and the absorbance at 415 nm was recorded.

Immunofluorescence staining

BMSCs were grown on chamber slides, fixed in 4% paraformaldehyde and then blocked in 1% BSA/PBS with 5% normal serum overnight at 4°C, and then incubated with primary antibody diluted in 1% BSA/PBS at 4°C overnight in a humid box. The slides were washed 3 times with PBS, incubated with FITC- or TRITC-coupled secondary antibodies diluted in 1% BSA/PBS for 40 min at R.T., washed 3 times with PBS to remove unbound antibodies, mounted with *SlowFade*® Gold antifade reagent with DAPI (Invitrogen, USA), sealed with nail polish and subjected for microscopy analysis. Photos were processed with Photoshop CS®.

Protein extraction, fractionation, Western blotting, and co-immunoprecipitation

Whole cell lysate was prepared by suspending the cells in 5 volumes of suspension buffer (0.1 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1mM DTT, pH 8.0, protease inhibitors), and then adding 5 volumes of Laemmli buffer (0.1 M Tris-HCl, pH 7.0, 4% SDS, 20% glycerol, 1 mM DTT, protease inhibitors) and boiling for 5 min.

Cells were fractionated as described (Mendez and Stillman, 2000). Briefly, the cells were suspended in 100 µl ice-cold buffer A (10 mM HEPES, pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT, protease inhibitors). After the addition of 0.1% Triton X-100, the cell suspension was mixed gently, incubated on ice for 5 min and centrifuged at 1300 x g at 4°C for 4 min. The supernatant (S1) was transferred to a new tube and clarified by high-speed centrifugation (12000 x g, 10 min, 4°C). The remaining nuclei pellet (P1) was washed once with 100 µl buffer A and then resuspended in 100 µl buffer A supplemented with 1 mM $CaCl_2$ and 2 units of micrococcal nuclease, and incubated at 37°C for 15 min. The reaction was stopped by adding 1 mM EGTA and the suspension was then centrifuged at 1300 x g at 4°C for 4 min. The resultant pellet was resuspended in 100 µl of buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT) and centrifuged at 1700 x g at 4°C for 4

min. The supernatant (S2'), containing all the soluble components of the nucleoplasm and chromatin-bound proteins, was transferred to another tube; the remaining pellet (P2'), containing all the nuclear matrix components, was suspended in 100 µl Laemmli buffer and boiled for 5 min.

Western blotting was performed as described previously (Liu et al., 2005). Relative band intensity was measured by Image J® and normalized to corresponding wild-type or untreated controls as indicated.

For statistical analysis, at least three independent immunoblots were quantified and student T test was used for *P* values.

For co-immunoprecipitation, cells were lysed into pre-chilled RIPA buffer containing 300 mM or 500 mM NaCl and protease inhibitors. Primary antibodies or appropriate control IgGs were added to the lysate and incubated for 2 h on a rocking platform at 4°C before Agarose beads were added and incubated O/N. The beads were washed twice with RIPA buffer, resuspended into Laemmli buffer and boiled, and protein suspension was collected by centrifugation and stored for further analysis.

In vitro SIRT1 deacetylation assay

SIRT1 deacetylation assay on fluorophore-conjugated synthetic p53 peptide was performed with SIRT1 Fluorimetric Drug Discovery Kit (Biomol, Hamburg, Germany) according to the manufactory's instruction. Cells were fractionated as described above except that protease inhibitors were NOT included and the nuclear matrix fraction was suspended into the assay buffer provided by the supplier. Different cell fractions or recombinant human lamin A (rhLamin A, Diatheva, Italy) were added in the reaction mix of SIRT1 Fluorimetric Drug Discovery Kit, incubated for 20 min at 37°C, and then the reaction was stopped and fluorescent signal was detected. SIRT1 deacetylase activity on native target was assayed using SIRT1 assay Kit from Sigma (USA). Constructs encoding FLAG-p53 and HA-p300 were co-transfected into HEK293 cells; FLAG-p53 was immunoprecipitated by anti-FLAG M2

Agarose (Sigma) followed by elution with FLAG peptide (Sigma). Purified acetyl FLAG-p53 was incubated with recombinant human SIRT1 (rhSIRT1) and NAD+ for 30 min at 37°C, in the presence or absence of rhLamin A or resveratrol. The acetylation level of FLAG-p53 was determined by Western blotting with pan anti acetyl lysine antibodies.

EXAMPLES

Following are examples that illustrate embodiments and procedures for practicing the invention. The examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1 - SIRT1 INTERACTS WITH LAMIN A WHILE PRELAMIN A OR PROGERIN JEOPARDIZES THE INTERACTION

To determine the potential involvement of SIRT1 in progeria, the potential interaction between lamin A and SIRT1 is examined by co-immunoprecipitation in HEK293 cells expressing ectopic FLAG-tagged SIRT1.

Lamin A was pulled down in the anti-FLAG immunoprecipitates, while FLAG-SIRT1 was detected in the anti-lamin A/C immunoprecipitates (**Figure 1A**). The interaction between endogenous SIRT1 and lamin A/C was confirmed in HEK293 cells, bone marrow stromal cells (BMSCs), and mouse embryonic fibroblasts (MEFs), where anti-SIRT1 immunoprecipitates pulled down lamin A and vice versa (**Figures 1B, 6A, 6B**).

Immunofluorescence confocal microscopy showed that much of nuclear SIRT1 co-localized with nucleoplasmic lamin A/C in the nuclear interior in human fibroblasts (**Figure 1C**). Consistently, ectopic EGFP-SIRT1 and DsRed-lamin A co-existed in the nuclear interior (**Figure 1D**). This interaction seems specific to nuclear SIRT1 as neither cytoplasmic SIRT1 nor mitochondrial SIRT5 was detected in the anti GFP-lamin A immunoprecipitates (**Figure 6C**). In addition, SIRT1 physically interacts with lamin A as recombinant human SIRT1 (rhSIRT1) was pulled down by recombinant human lamin A (rhLamin A) in a test tube (**Figure 1E**).

Alternative splicing of *LMNA* gives rise to different A-type lamins, of which lamin A and C are the most abundant (Lin and Worman, 1993). Lamin A and C share the first 566 amino acids; lamin A has a specific 98-amino-acid carboxyl tail, and lamin C has a unique 6-arnino-acid carboxyl tail (Liu and Zhou, 2008).

Although the level of lamin C was much higher than A in HEK293 cells, lamin C was hardly detected in the anti-SIRT1 immunoprecipitates (**Figure 1B**), indicating that lamin A likely interacts with SIRT1 via its C-terminal domain. This was further confirmed by co-immunoprecipitation in HEK293 cells expressing FLAG-SIRT1 together with either lamin A or lamin C. As shown in **Figure 1F**, lamin A was detected in the anti-FLAG-SIRT1 immunoprecipitates, whereas lamin C was negligible.

It is widely accepted that the unprocessed C-terminal tail in progerin or prelamin A is responsible for the progeroid features in HGPS and progeria mouse models. Given that lamin A interacts with SIRT1 via its C-terminal domain, this Example examines whether the interaction between SIRT1 and prelamin A or progerin is reduced compared to lamin A. Co-immunoprecipitation was performed in HEK293 cells expressing FLAG-SIRT1 together with one of the A-type lamins, i.e., wild-type lamin A, prelamin A or progerin.

As shown in **Figures 1G, 1H**, significantly less prelamin A and progerin were pulled down by anti-FLAG antibodies compared with lamin A, though comparable or higher levels of FLAG-SIRT1 and prelamin A/progerin were present in the input. The results indicate that SIRT1 preferentially interacts with lamin A whereas prelamin A or progerin has significantly reduced association with SIRT1.

EXAMPLE 2 - SIRT1 IS MISLOCALIZED IN PROGERIA CELLS

Lamin A is one of the major components of NM. This Example investigates the association of SIRT1 with the NM by subcellular fractionation. *SIRT1*$^{-/-}$ cells were utilized as a negative control for the specific staining of SIRT1 protein. KAP-1 (KRAB-associated protein 1,

also known as Trim28 or Tif1β) and MCM3 proteins served as positive controls for the purity of the subcellular fractionation.

KAP-1 is a heterochromatin factor and was reported to be associated with the majority of the micrococcal nuclease-resistant fraction in the nucleus (Goodarzi et al., 2008; Ryan et al., 1999). MCM3 protein is important in preventing excessive DNA replication during S phase and is predominantly associated with chromatin (Mendez and Stillman, 2000).

As expected, Kap-1 was resistant to MNase digestion and remained in the NM fraction (P2') whereas the majority of Mcm3 was released into the nucleoplasmic and chromatic fraction (S2') after MNase treatment (**Figure 7A**). Consistent with its interaction with lamin A, SIRT1 protein was enriched in the NM fraction (P2') in MEFs (**Figure 7B**).

Since prelamin A has less binding capacity to SIRT1 compared with lamin A and SIRT1 is highly expressed in stem cells (Saunders et al., 2010), this Example also examines SIRT1 localization in *Zmpste24*$^{-/-}$ cells by subcellular fractionation in multipotent BMSCs.

NM-associated SIRT1 was largely reduced in *Zmpste24*$^{-/-}$ BMSCs compared to wild type controls, though total nuclear proportion of SIRT1 was comparable (**Figures 2A, 2B**). The reduction in NM-associated SIRT1 appears specific, because Sirt6, CBP acetyltransferase and Foxo3a were not significantly affected in *Zmpste24*$^{-/-}$ cells.

The NM-associated SIRT1 was also reduced in HGPS dermal fibroblasts, including HGADFN143, HGADFN188, HGADFN164 and HGADFN122, compared to either healthy F2-S fibroblasts or dermal fibroblasts harboring non-progeria *LMNA* mutations, i.e., R453W in Emery Dreifuss Muscular Dystrophy (EDMD), R482W in Familial Lipodystrophy (FLPD) and R401C in EDMD (Liu and Zhou, 2008) (**Figures 7C, 7D**).

Though total nuclear level of SIRT1 was variable in different HGPS cell lines, the percentage of NM-associated SIRT1 was consistently reduced. Moreover, ectopic expression of prelamin A and progerin caused remarkable disassociation of SIRT1 from the NM, while the

nuclear level of SIRT1 was hardly affected in HEK293 cells (**Figures 2C, 2D**). The results indicate that prelamin A or progerin compromises the proper NM localization of SIRT1.

To further assess the functional significance of mislocalization of SIRT1, this Example examines SIRT1 downstream pathway(s) in progeria cells. SIRT1 deacetylates Foxo3a and upregulates its transcriptional activity, thus promoting expression of antioxidant enzymes such as MnSOD and catalase in response to oxidative stress (Brunet et al., 2004).

Consistent with the mislocalization of SIRT1, Foxo3a was hyper-acetylated in *Zmpste24*$^{-/-}$ BMSCs and the level of catalase and Gadd45α was reduced by approximately 40% in *Zmpste24*$^{-/-}$ mice relative to wild-type controls (**Figures 2E-F**). The increase in Foxo3a acetylation is likely the result of decreased SIRT1 deacetylase activity *in vivo,* as neither total nuclear SIRT1 level nor NM association of CBP, the acetyltransferase for Foxo3a, was changed in *Zmpste24*$^{-/-}$ BMSCs (**Figures 2A-B**). Ectopic expression of either prelamin A or progerin increased the acetylation of FOXO3A, and reduced the expression of catalase, MnSOD and GADD45α in HEK293 cells (**Figure 2G**).

EXAMPLE 3 - RESVERATROL ENHANCES THE BINDING OF SIRT1 TO LAMIN A AND STIMULATES ITS DEACETYLASE ACTIVITY IN A LAMIN A-DEPENDENT MANNER

The NM-localization of SIRT1 and the association of NM with HDAC deacetylase activity (Fey et al., 1991) suggest that NM might contain potential SIRT1 activators. To test this hypothesis, *in vitro* deacetylase activity of rhSIRT1 by a BioMol® SIRT1 Fluorimetric Drug Discovery Kit (BSDK) was quantified in the presence or absence of NM derived from wild-type or *Zmpste24*$^{-/-}$ BMSCs.

Surprisingly, the deacetylase activity of rhSIRT1 was enhanced approximately 3-fold in the presence of NM from wild-type BMSCs compared with the control without NM or with cytoplasmic fraction (**Figure 7E**), suggesting the existence of potential SIRT1 activator(s) on the NM. In contrast, the NM from the *Zmpste24*$^{-/-}$ BMSCs showed a significantly reduced stimulatory effect on rhSIRT1 deacetylase activity.

This Example further investigates whether lamin A acts as an activator of SIRT1. In mammalian cells, lysine acetyltransferase p300 and SIRT1 mediate the acetylation and deacetylation of p53 on residue K382 (Gu and Roeder, 1997). BSDK utilizes fluorophore-conjugated acetyl p53 peptide as target (see Materials and Methods). As shown in **Figure 3A**, in the presence of rhLamin A, the deacetylase activity of rhSIRT1 on acetyl p53 peptide was increased in a dose-dependent manner. Lamin A-stimulated SIRT1 deacetylase activity was completely abolished by SIRT1 inhibitor Suramin.

To further test the effect of rhLamin A on the native target of SIRT1, acetyl p53 was purified by anti-FLAG immunoprecipitation in HEK293 cells ectopically expressing FLAG-p53 and p300, and SIRT1 deacetylation assay was performed as described in Materials and Methods.

As shown in Figures **3B** and **S2F**, around 20% decrease in FLAG-p53 acetylation level was observed in the presence of rhLamin A-rhSIRT1 complex compared with rhSIRT1 only, suggesting that lamin A serves as an activator of SIRT1.

Resveratrol, which might be a potential SIRT1 activator, has been reported to enhance healthspan in a range of age-related diseases. However several independent studies found that resveratrol activates SIRT1 towards the fluorophore-conjugated synthetic p53 peptide rather than unconjugated native targets (Borra et al., 2005; Burnett et al., 2011; Dai et al., 2010; Kaeberlein et al., 2005; Pacholec et al., 2010).

This Example shows that resveratrol does not directly activate SIRT1 deacetylase activity using native full-length FLAG-p53 as a substrate (**Figure 7F**). Surprisingly, in the presence of rhLamin A, resveratrol activated SIRT1 in a lamin A dose-dependent manner (**Figures 3B, 7F**). Further examination revealed that resveratrol enhanced the binding of rhSIRT1 to rhLamin A in the test tube (**Figure 3C**) and the binding of FLAG-SIRT1 to lamin A in HEK293 cells (**Figures 3D, 3E**) by co-immunoprecipitation.

In addition to acetyl p53, H3K9ac is another substrate of SIRT1 deacetylase (Wang et al., 2008). This Example further investigates the effects of resveratrol on H3K9ac in the wild-type,

SIRT1$^{-/-}$ or *Lmna* null cells. As shown in **Figure 3F**, treatment with resveratrol downregulated the level of H3K9ac in wild-type cells in a dose-dependent manner, while this effect was completely abrogated in *SIRT1*$^{-/-}$ or *Lmna*$^{-/-}$ cells. Anti-H3K9ac immunofluorescence staining further confirmed the lamin A-dependent activation of SIRT1 by resveratrol (**Figure 3G**).

As SIRT1 interacts with lamin A and thus associates with NM and resveratrol increases the binding between lamin A and SIRT1, this Example also examines whether resveratrol enhances NM association of SIRT1. As shown in **Figures 7G-H**, in wild-type and *Zmpste24*$^{-/-}$ BMSCs incubated with different concentrations of resveratrol, NM-associated SIRT1 was increased compared to untreated controls. The ability of resveratrol to stimulate the NM association of SIRT1 was also observed in test tube. When equal amounts of rhSIRT1 were incubated with the insoluble NM fraction from either wild-type or *Zmpste24*$^{-/-}$ cells suspended in BSDK assay buffer, significant less NM-bound rhSIRT1 was found in precipitates from *Zmpste24*$^{-/-}$ NM relative to wild-type NM precipitates in the absence of resveratrol (**Figure 3H**). Significantly, the presence of resveratrol enhanced the association of rhSIRT1 with NM derived from both wild-type and *Zmpste24*$^{-/-}$ cells. Consistently, rhSIRT1 level in the supernatant underwent a compensatory reduction (**Figure 3H**). Collectively, these data indicate that resveratrol could enhance the interaction between lamin A and SIRT1 to increase the NM association of SIRT1 and thus activates SIRT1.

EXAMPLE 4 - RESVERATROL TREATMENT RESCUES ASC DECLINE IN *ZMPSTE24*$^{-/-}$ MICE

As resveratrol enhances SIRT1 deacetylase activity by increasing its binding to lamin A, this Example examines the effects of resveratrol treatment on the early senescence in *Zmpste24*$^{-/-}$ MEFs. However, no obvious difference in β-galactosidase activity was observed between resveratrol-treated and saline-treated *Zmpste24*$^{-/-}$ MEFs (**Figure 8A**), and resveratrol did not reduce the elevated levels of $p16^{Ink4a}$ in *Zmpste24*$^{-/-}$ MEFs (**Figure 8B**). As SIRT1 is more

highly expressed in stem cells than in somatic differentiated cells and it is critical for maintaining stem cell self-renewal and function (Han et al., 2008; Lee et al., 2011; Saunders et al., 2010), we then tested effects of resveratrol on BMSCs.

Progerin and prelamin A have been previously linked to defects in mesenchymal stem cells (MSCs) and in hair follicle progenitor cells in *Zmpste24*$^{-/-}$ mice (Espada et al., 2008; Scaffidi and Misteli, 2008). Consistently, the number of BMSCs was significantly reduced in *Zmpste24*$^{-/-}$mice compared with wild-type controls at 4 months of age (**Figures 9A**). *Zmpste24*$^{-/-}$ BMSCs in culture showed compromised colony-forming capacity (**Figure 9B**), reduced proliferation (**Figure 9C**), and a dramatically increased cellular senescence (**Figures 9D**). Similarly, an early decline in mononucleated cells (MNCs) and hematopoietic stem cells (HSCs, Lineage^{-}Flt3^{-} Sca-l^{+}cKit^{high}) was observed in *Zmpste24*$^{-/-}$ mice (**Figures 9E-G**), such that by 4 months of age HSC levels fell to less than half of that of wild-type controls. HSC transplantation experiments showed that the self-renewal defects were cell-intrinsic (**Figure 9H**). Interestingly, resveratrol enhanced the colony-forming capacity in *Zmpste24*$^{-/-}$ BMSCs in a dose-dependent manner (**Figures 4A-B**). The treatment also increased the binding of SIRT1 to prelamin A and the expression of Gadd45α and catalase (**Figures 4C-D**). Moreover, the rescue effect of resveratrol is SIRT1-dependent, as knocking down SIRT1 attenuated its effect on *Zmpste24*$^{-/-}$ BMSCs (**Figures 4D-F**). Knocking down SIRT1 abolished the stimulating effect of resveratrol on the expression of Gadd45α and catalase (**Figure 4D**). In addition, knockdown of SIRT1 decreased colony-forming capacity (**Figures 4E-F**) and ectopic SIRT1 increased the colony-forming capacity of *Zmpste24*$^{-/-}$ BMSCs to levels comparable to that of wild-type BMSCs (**Figures 4G-I**). These data indicate that BMSC decline in *Zmpste24*$^{-/-}$ mice is attributable to impaired SIRT1 function which can be rescued by resveratrol.

EXAMPLE 5 - RESVERATROL ALLEVIATES PROGEROID FEATURES AND EXTENDS LIFESPAN IN *ZMPSTE24*$^{-/-}$ MICE

As the SIRT1-dependent rescue of BMSC colony-forming capacity *in vitro*, this Example examines whether resveratrol could rescue the BMSC defects in *Zmpste24*$^{-/-}$ mice *in vivo*.

Briefly, resveratrol was supplemented at a concentration of 20 μg/ml in drinking water. Four months after treatment, BMSCs were collected for examination and comparison between resveratrol-treated and vehicle-treated *Zmpste24*$^{-/-}$ mice.

As shown in **Figures 5A** and **5B**, BMSC colony-forming efficiency was significantly increased in the resveratrol-treated group. Concomitantly, resveratrol treatment enhanced the level of catalase whereas it decreased the level of acetyl p53 and H3K9 in BMSCs (**Figure 5C**). Resveratrol-treatment also rescued the early decline in HSCs (**Figure 5C**). Moreover resveratrol-treatment ameliorated progeroid features in *Zmpste24*$^{-/-}$ mice. As shown by micro-CT analyses, the trabeculae in *Zmpste24*$^{-/-}$ mice appeared to be thinner and more widely spaced. After 4 months of treatment, resveratrol increased the trabecular thickness, improved the structural organization and increased bone mineral density (**Figures 5D-E**). In addition, resveratrol-treatment also significantly slowed down the body weight loss, compared to vehicle-treated controls (**Figures 5F-G**). Most importantly, the median survival was extended from 20 weeks in vehicle-treated *Zmpste24*$^{-/-}$ mice to 27 weeks in resveratrol-treated *Zmpste24*$^{-/-}$ mice (**Figure 5H**). By 26 weeks after birth, 95% of *Zmpste24*$^{-/-}$ mice had died, whereas nearly 60% of resveratrol-treated animals were still alive. The maximum lifespan (mean lifespan of the longest-lived 10% of the animals) was prolonged from 27.5 weeks in vehicle-treated to 33.5 weeks in resveratrol-treated *Zmpste24*$^{-/-}$ mice (**Figure 5I**).

Resveratrol rescues SIRT1-dependent decline in adult stem cell numbers and alleviates progeroid features in laminopathy-based progeria. Mice lacking Zmpste24, a metalloproteinase responsible for prelamin A processing, recapitulate many of the HGPS features, including accelerated cellular senescence and dysfunctional adult stem cells (ASCs). SIRT1 directly interacts with lamin A, and thus, localizes on the nuclear matrix (NM). The association of SIRT1 with lamin A and NM enhances its deacetylase activity.

Compared with lamin A, prelamin A and progerin have significantly decreased interaction with SIRT1 *in vivo* and reduced activation of SIRT1 deacetylase activity, leading to rapid depletion of ASCs in laminopathy-based progeria mice. Resveratrol activates SIRT1 via increasing the binding of SIRT1 to A-type lamins and enhancing its NM association. Resveratrol treatment rescues ASC decline in a SIRT1-dependent manner, slows down body weight loss, improves trabecular bone structure and mineral density, and significantly extends the lifespan of *Zmpste24*$^{-/-}$ mice. The present invention shows that lamin A is an activator of SIRT1 and resveratrol directly activates SIRT1 in a lamin A-dependent manner. Further, the association between conserved SIRT1 longevity pathway and progeria indicates that the stem cell-based and SIRT1 pathway-dependent therapeutic strategies can be useful for treatment of progeria.

SIRT1 interacts with lamin A and associates with the NM; lamin A serves as an activator of SIRT1. SIRT1 interacts weakly with prelamin A or progerin, and SIRT1 abundance and activity in the NM is significantly reduced in progeria cells. Resveratrol directly activates SIRT1 through enhancing the binding between SIRT1 and A-type lamins and increasing the association of SIRT1 with the NM, which in turn rescues the defective BMSCs, ameliorates progeroid phenotypes, and extends the lifespan of *Zmpste24*$^{-/-}$ mice.

The present invention shows that lamin A directly interacts with SIRT1 and thus enhances deacetylase activity of SIRT1, using either AMC-conjugated synthetic peptide or native full-length acetyl p53. It is postulated that lamin A interacts with SIRT1 through its C-terminus, as the interaction between lamin C and SIRT1 is minimal. The unprocessed C-terminus in prelamin A or progerin may interfere with their binding with SIRT1, and therefore, reduce SIRT1 association on NM, leading to jeopardized deacetylase activity in progeria cells. Resveratrol enhances the binding of SIRT1 to A-type lamins and thus activates SIRT1 deacetylase activity.

Although many of the *in vivo* benefits of resveratrol are SIRT1-dependent (Baur, 2010), emerging evidence indicates that it also activates AMPK independent of SIRT1 (Canto et al., 2009; Gledhill et al., 2007; Hawley et al., 2010; Park et al., 2012). It was shown that low doses of resveratrol (≤ 25 μM) activates AMPK in a SIRT1-dependent manner, while high doses (≥ 50 μM) activate AMPK independent of SIRT1 (Baur et al., 2006; Price et al., 2012; Sun et al., 2007). In the present invention, low doses (2-10 μM) of resveratrol were used and activation of AMPK was not observed; therefore, it is postulated that the beneficial effects of resveratrol on BMSCs are primarily attributable to the activation of SIRT1.Interestingly, although resveratrol rescues BMSC decline in a SIRT1-dependent manner, ameliorates progeroid phenotypes and extends the lifespan of *Zmpste24*$^{-/-}$ mice, it does not rescue cellular senescence in *Zmpste24*$^{-/-}$ MEFs, suggesting that resveratrol treatment may affect somatic cells and ASCs differently.

Accordingly, it has been reported that resveratrol inhibits proliferation of somatic cells of different origins and in various cancer cell lines (Sgambato et al., 2001). As the effects of resveratrol are pleiotropic (Harikumar and Aggarwal, 2008) and involve both sirtuin-dependent and independent pathways, it seems plausible that resveratrol could influence sirtuin and non-sirtuin pathways differently in ASCs and somatic cells.

Given the fact that SIRT1 expression is much higher in stem cells compared to that in somatic cells (Saunders et al., 2010), the different effects of resveratrol on BMSCs and MEFs may also lie in the difference in level of SIRT1 expression. Indeed, the effect of resveratrol on BMSCs is SIRT1-dependent and ectopic SIRT1 enhances colony-forming capacity in both wild-type and *Zmpste24*$^{-/-}$ BMSCs. It is likely that the lifespan extension in *Zmpste24*$^{-/-}$ mice by resveratrol is attributable, at least in part, to the rescue of SIRT1-dependent ASC decline, e.g. BMSCs and HSCs etc. Since SIRT1 affects multiple pathways (Lavu et al., 2008; Smith et al., 2008), it is not clear which downstream targets of SIRT1 influence the maintenance of BMSCs in *Zmpste24*$^{-/-}$ mice. However, Foxo3a-mediated oxidative detoxification would be one potential candidate because directly scavenging ROS level via N-acetyl Cysteine (NAC) rescued

the decline in BMSCs and HSCs and extended lifespan to a similar extent as resveratrol did (**Figure 10**). Although lifespan extension in *C. elegans* and *Drosophila* by ectopic Sir2 was recently called into question, SIRT1 deficiency in mammals may affect metabolic and transcriptional adaptation essential for life in response to stress (Burnett et al., 2011; Chalkiadaki and Guarente, 2012; Houtkooper et al., 2012; Lombard et al., 2011; Viswanathan and Guarente, 2011). In this regard, it should be emphasized that lifespan extension in progeroid mice by resveratrol may be the consequence of elevated SIRT1-dependent physiological and metabolic functions necessary for healthspan that are severely compromised in *Zmpste24*$^{-/-}$ mice. Resveratrol prolongs lifespan and delays aging-related phenotypes in a short-lived fish strain (Valenzano et al., 2006).

The present invention shows that (i) resveratrol activates SIRT1 via increasing its binding to lamin A; (ii) a perturbed interaction between SIRT1 and A-type lamins compromises SIRT1 function, leading to impaired ASC population which contributes, at least partially, to some of the phenotypes associated with HGPS; (iii) resveratrol facilitates the interaction between SIRT1 and A-types lamins to activate SIRT1, and therefore, rescuing ASC decline, ameliorating progeroid phenotypes and extending lifespan in a mouse model of progeria. The present invention shows that resveratrol directly targets SIRT1. Also, resveratrol and other SIRT1 activators can be used in the treatment of HGPS.

All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

The terms “a” and “an” and “the” and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated

herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (*e.g.*, all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (*e.g.*, a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

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Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M., and Sinclair, D. (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature *430*, 686-689.

Zhao, W., Kruse, J.P., Tang, Y., Jung, S.Y., Qin, J., and Gu, W. (2008). Negative regulation of the deacetylase SIRT1 by DBC1. Nature *451*, 587-590.

REPRESENTATIVE CLAIMS

What is claimed is:

1. A method of modulating the deacetylase activity of SIRT1 in a mammalian cell, the method comprising modifying the binding affinity of lamin A to SIRT1 via an interaction modifying compound.

2. The method of claim 1, wherein the deacetylase activity of SIRT1 is increased by an increased binding affinity of lamin A to SIRT1.

3. The method of claim 1, wherein the deacetylase activity of SIRT1 is decreased by a decreased binding affinity of lamin A to SIRT1.

4. The method of claim 1, wherein the interaction modifying compound comprises resveratrol, wherein resveratrol increases the binding affinity of lamin A to SIRT1.

5. A method for increasing in a mammalian cell the deacetylase activity of SIRT1, the method comprising contacting the cell with a lamin A peptide in an amount effective to increase the deacetylase activity of SIRT1.

6. The method of claim 5, wherein the lamin A peptide comprises the amino acid sequence of SEQ ID NO:2, or an analog thereof.

7. The method of claim 5, wherein the lamin A peptide comprises the carboxyl domain of lamin A, or an analog thereof.

8. The method of claim 5, wherein the lamin A peptide comprises amino acids 567-646 of SEQ ID NO:2, or a fragment thereof.

9. The method of claim 8, wherein the fragment is from about 3 amino acids to about 50 amino acids in length.

10. The method of claim 9, wherein the fragment is an analog.

11. A method for treating a disease or condition in which modulated SIRT1 deacetylase activity is beneficial, comprising administering to a subject in need of such treatment, an effective amount of an agent that modulates SIRT1 deacetylase activity.

12. The method of claim 11, wherein the agent increases SIRT1 deacetylase activity.

13. The method of claim 12, wherein the agent is a carboxyl terminal peptide of lamin A, or analog thereof.

14. The method of claim 12, wherein the increased SIRT1 deacetylase activity results in increased number of bone marrow stromal cells.

15. The method of claim 12, wherein the increased SIRT1 deacetylase activity results in increased number of hematopoietic stem cells.

16. The method of claim 11, wherein the agent decreases SIRT1 deacetylase activity.

17. The method of claim 16, wherein the disease or condition being treated is neoplasia.

18. A method of screening for agents that modulate SIRT1 deacetylase activity, wherein the method comprises:

contacting a candidate molecule with cells expressing SIRT1 in a test sample;

determining deacetylase activity in the test sample; and

selecting the candidate molecule as the agent that modulates SIRT1 deacetylase activity if said molecule changes the level of SIRT1 deacetylase activity in the test sample.

19. The method according to claim 18, wherein the candidate molecule is a fragment of the lamin A carboxyl domain, or an analog thereof.

20. The method according to claim 18, wherein the candidate molecule is a compound that enhances the binding of lamin A to SIRT1.

21. The method according to claim 18, wherein the candidate molecule is a compound that modulates lamin A protein level.

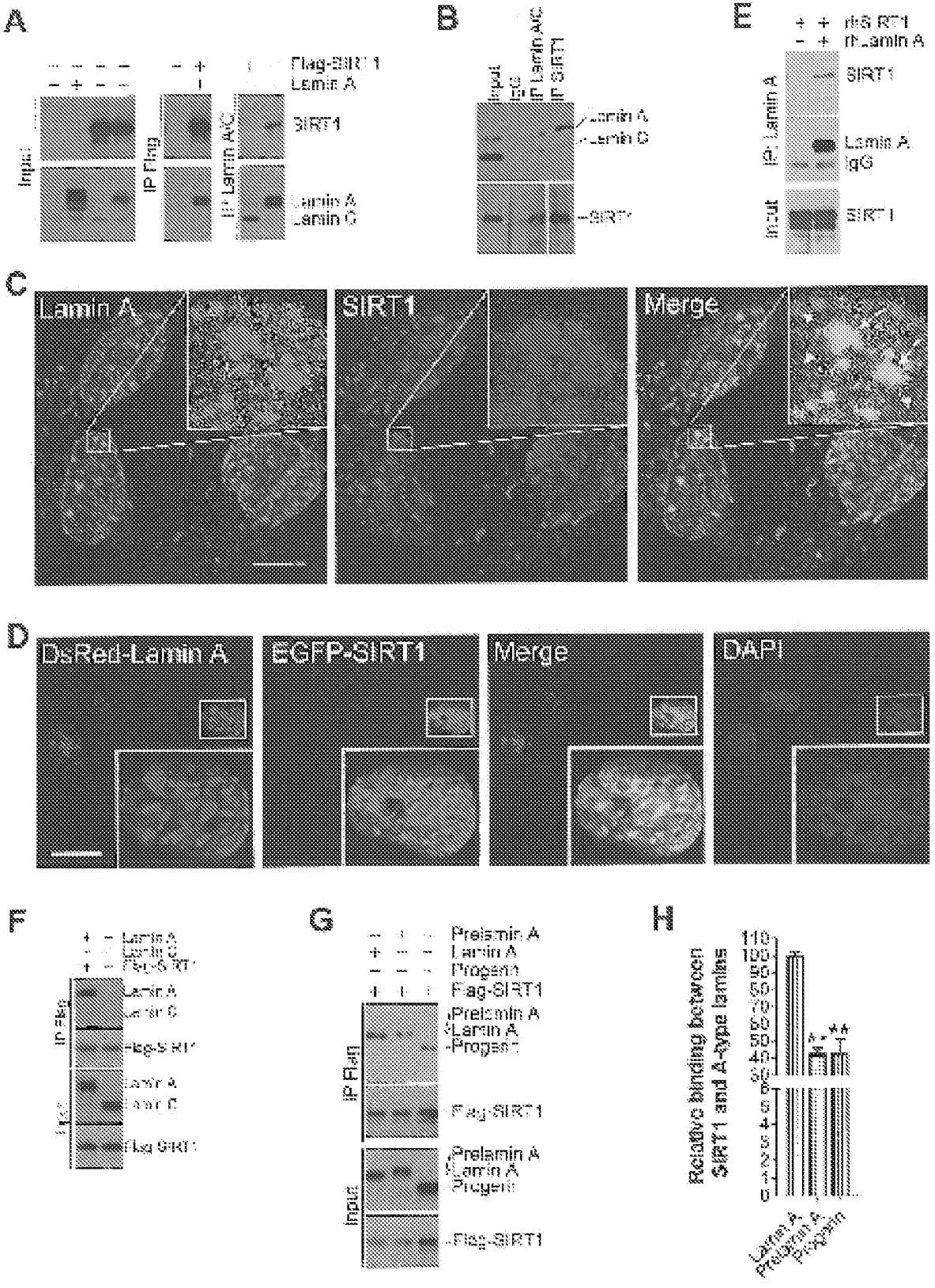
A
Flag-SIRT1
Lamin A
Input
IP Flag
IP Lamin A/C
SIRT1
Lamin A
Lamin C
B
Input
IgG
IP Lamin A/C
IP SIRT1
Lamin A
Lamin C
SIRT1
E
rfloS RT1
rfl-Lamin A
IP: Lamin A
SIRT1
Lamin A
IgG
Input
SIRT1
C
Lamin A
SIRT1
Merge
D
DsRed-Lamin A
EGFP-SIRT1
Merge
DAPI
F
Lamin A
Lamin C
Flag-SIRT1
IP Flag
Input
G
Prelamin A
Lamin A
Progerin
Flag-SIRT1
IP Flag
Input
H
Relative binding between SIRT1 and A-type lamins
Lamin A
Progerin
Prelamin A

FIG. 1

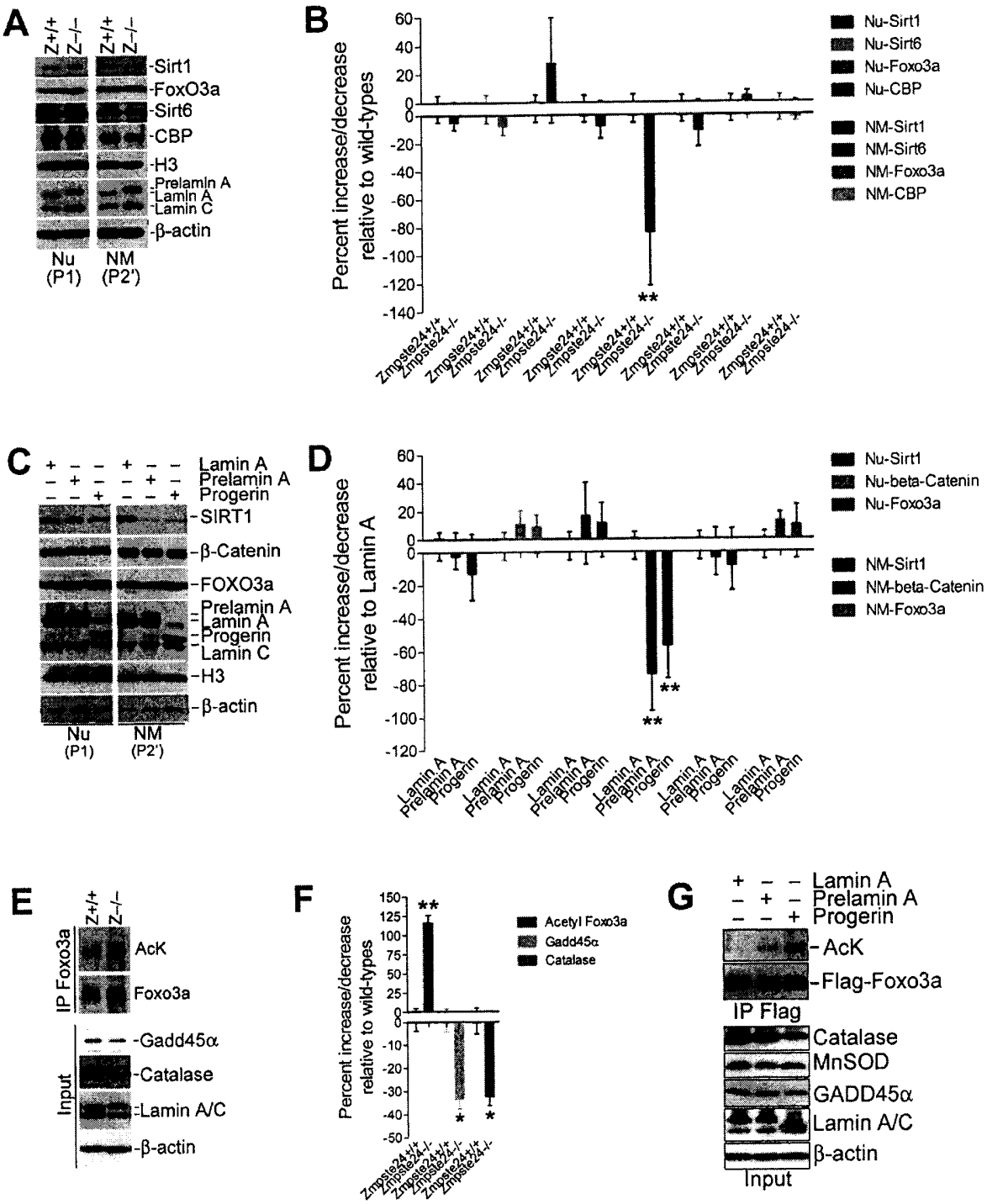
A
Z+/+
Z-/-
Sirt1
FoxO3a
Sirt6
CBP
H3
Prelamin A
Lamin A
Lamin C
β-actin
Nu (P1)
NM (P2')
B
Percent increase/decrease relative to wild-types
Nu-Sirt1
Nu-Sirt6
Nu-Foxo3a
Nu-CBP
NM-Sirt1
NM-Sirt6
NM-Foxo3a
NM-CBP
C
Lamin A
Prelamin A
Progerin
SIRT1
β-Catenin
FOXO3a
Prelamin A
Lamin A
Progerin
Lamin C
H3
β-actin
Nu (P1)
NM (P2')
D
Percent increase/decrease relative to Lamin A
Nu-Sirt1
Nu-beta-Catenin
Nu-Foxo3a
NM-Sirt1
NM-beta-Catenin
NM-Foxo3a
E
IP Foxo3a
AcK
Foxo3a
Input
Gadd45α
Catalase
Lamin A/C
β-actin
F
Percent increase/decrease relative to wild-types
Acetyl Foxo3a
Gadd45α
Catalase
G
Lamin A
Prelamin A
Progerin
AcK
Flag-Foxo3a
IP Flag
Catalase
MnSOD
GADD45α
Lamin A/C
β-actin
Input

FIG. 2

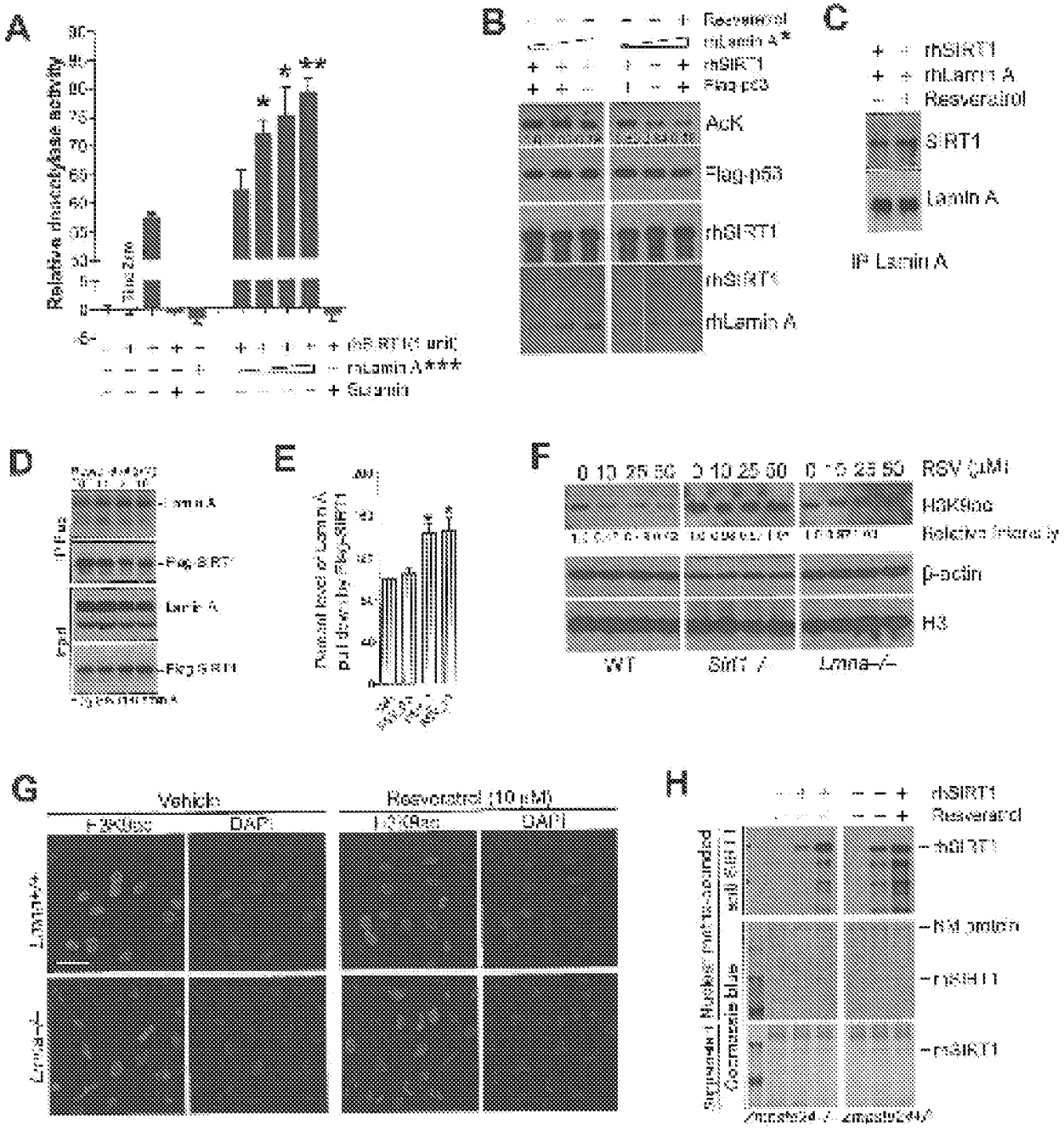
A
Relative deacetylase activity
Zinc Zero
rhSIRT1(5 unit)
rhLamin A***
Suramin
B
Resveratrol
rhLamin A*
rhSIRT1
Flag-p53
AcK
Flag-p53
rhSIRT1
rhSIRT1
rhLamin A
C
rhSIRT1
rhLamin A
Resveratrol
SIRT1
Lamin A
IP: Lamin A
D
Lamin A
Flag-SIRT1
Lamin A
Flag-SIRT1
IP: Flag
Input
E
Relative level of Lamin A pull down by Flag-SIRT1
F
0 10 25 50 0 10 25 50 0 10 25 50 RSV (μM)
H3K9ac
Relative Intensity
β-actin
H3
WT
Sirt1 -/-
Lmna-/-
G
Vehicle
Resveratrol (10 μM)
H3K9ac
DAPI
H3K9ac
DAPI
Lmna+/+
Lmna-/-
H
rhSIRT1
Resveratrol
rhSIRT1
NM protein
rhSIRT1
rhSIRT1
Supernatant
Nuclear matrix-associated
anti-SIRT1
Coomassie blue
Lmna+/+
Lmna-/-

FIG. 3

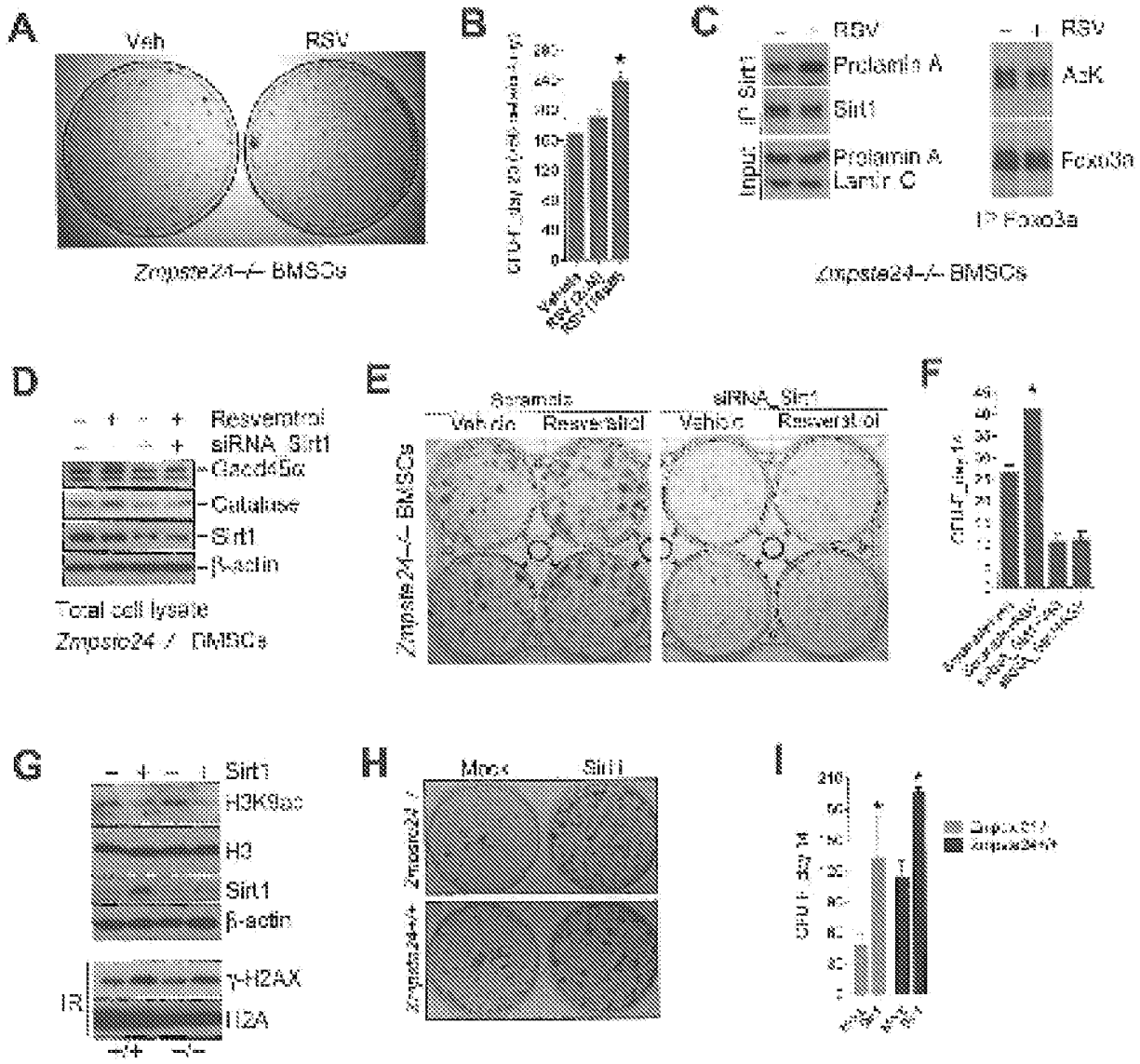
A
Veh
RSV
Zmpste24-/- BMSCs
B
C
RSV
Prelamin A
Sirt1
Prelamin A
Lamin C
Input
IP Sirt1
RSV
AcK
Foxo3a
IP Foxo3a
Zmpste24-/- BMSCs
D
Resveratrol
siRNA_Sirt1
Gadd45α
Catalase
Sirt1
β-actin
Total cell lysate
Zmpste24-/- BMSCs
E
Scramble
siRNA_Sirt1
Vehicle
Resveratrol
Vehicle
Resveratrol
Zmpste24-/- BMSCs
F
G
Sirt1
H3K9ac
H3
Sirt1
β-actin
IR
γ-H2AX
H2A
-/+
-/-
H
Mock
Sirt1
Zmpste24-/-
Zmpste24+/+
I

FIG. 4

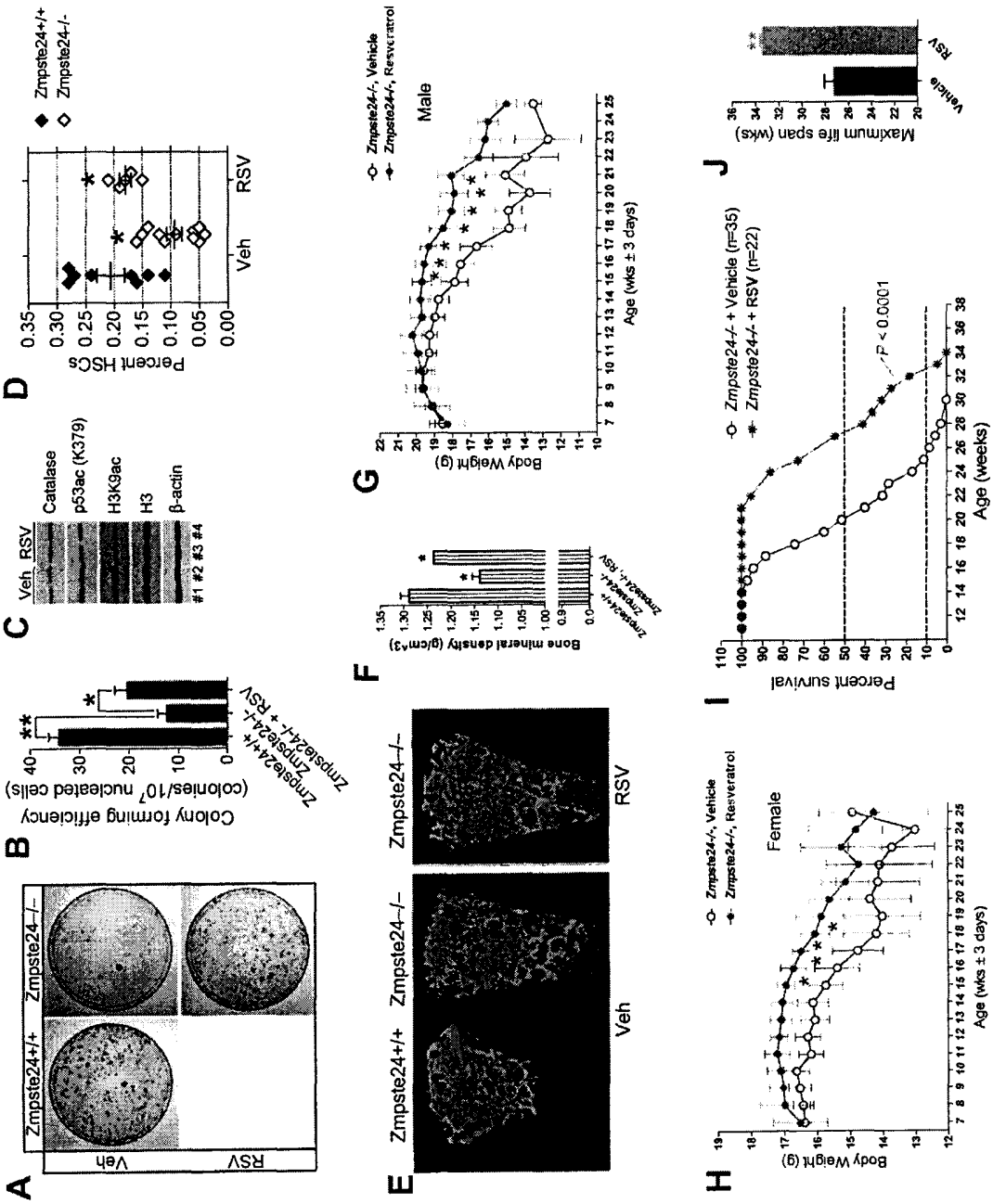
A
Zmpste24+/+
Zmpste24−/−
Veh
RSV
B
Colony forming efficiency
(colonies/10^7 nucleated cells)
Zmpste24+/+
Zmpste24−/−
Zmpste24−/− + RSV
C
Veh RSV
Catalase
p53ac (K379)
H3K9ac
H3
β-actin
#1 #2 #3 #4
D
Zmpste24+/+
Zmpste24−/−
Percent HSCs
Veh
RSV
E
Zmpste24+/+
Zmpste24−/−
Zmpste24−/−
Veh
RSV
F
Bone mineral density (g/cm^3)
G
Zmpste24−/−, Vehicle
Zmpste24−/−, Resveratrol
Male
Body Weight (g)
Age (wks ± 3 days)
H
Zmpste24−/−, Vehicle
Zmpste24−/−, Resveratrol
Female
Body Weight (g)
Age (wks ± 3 days)
I
Zmpste24−/− + Vehicle (n=35)
Zmpste24−/− + RSV (n=22)
P < 0.0001
Percent survival
Age (weeks)
J
Maximum life span (wks)
vehicle
RSV

FIG. 5

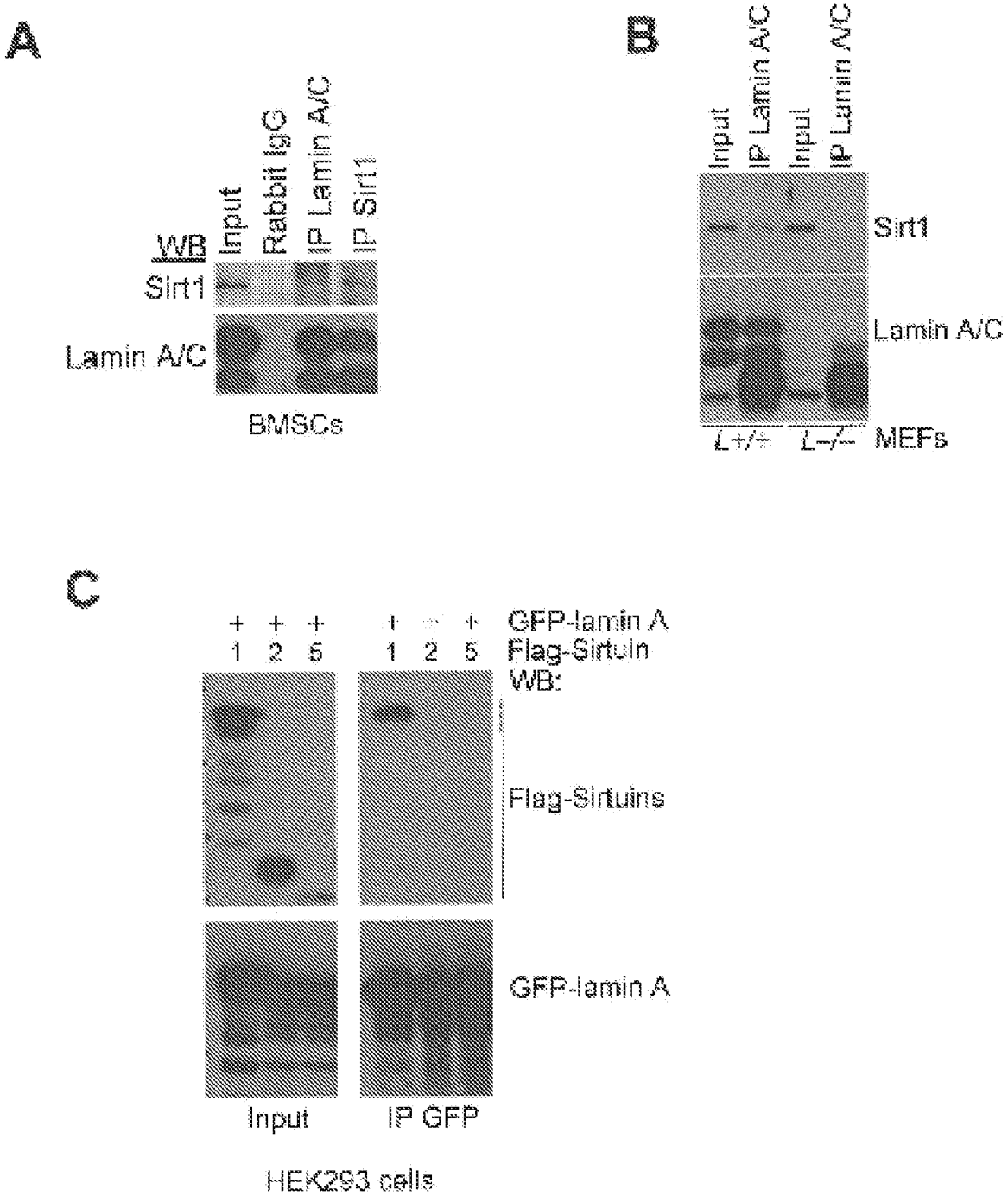
A
WB
Input
Rabbit IgG
IP Lamin A/C
IP Sirt1
Sirt1
Lamin A/C
BMSCs
B
Input
IP Lamin A/C
Input
IP Lamin A/C
Sirt1
Lamin A/C
L+/+
L-/-
MEFs
C
+ + + + - + GFP-lamin A
1 2 5 1 2 5 Flag-Sirtuin
WB:
Flag-Sirtuins
GFP-lamin A
Input
IP GFP
HEK293 cells

FIG. 6

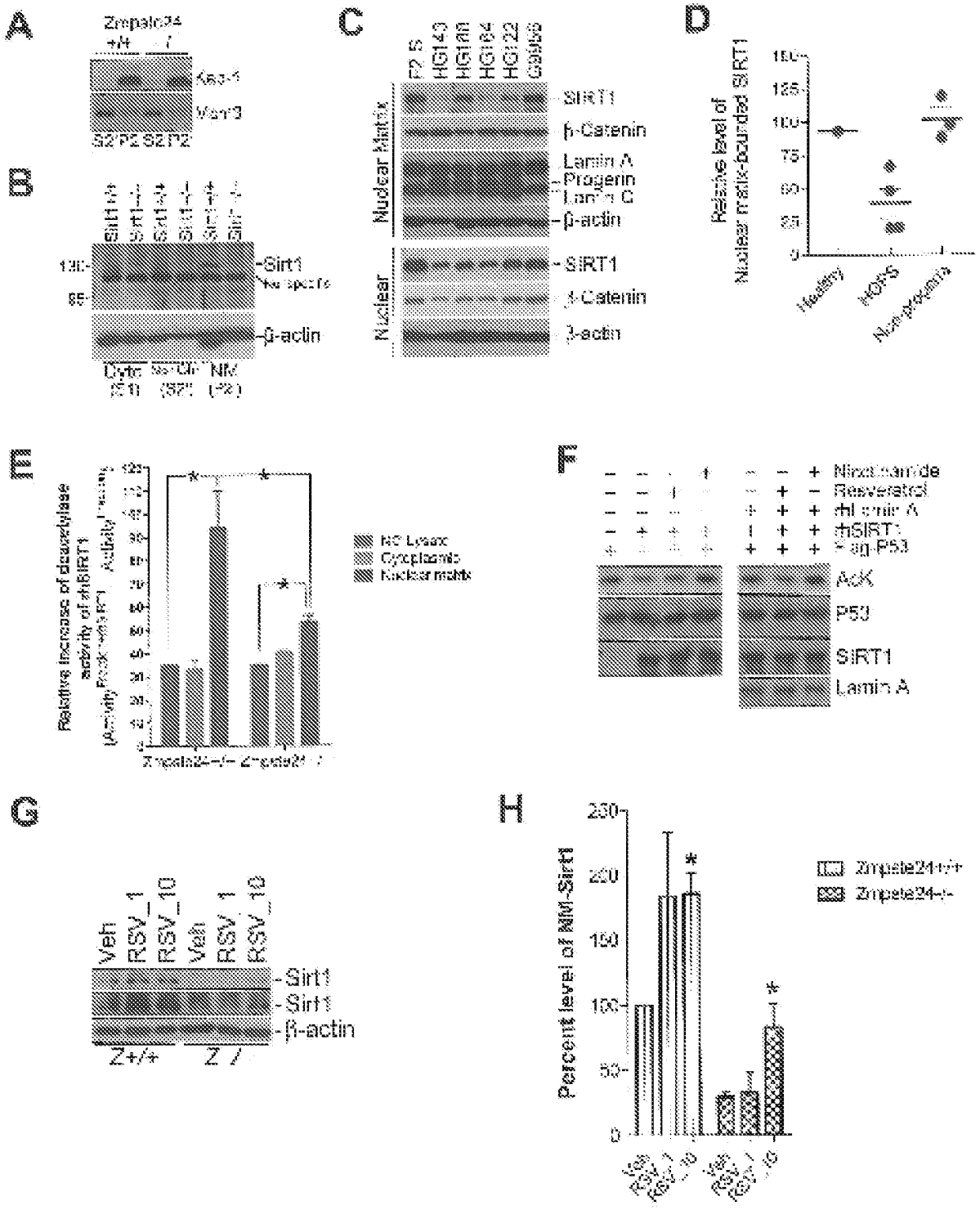
A
Zmpste24
+/+
-/-
Klotho
Vav3
P2
B
Sirt1+/+
Sirt1-/-
-Sirt1
-β-actin
C
Nuclear Matrix
Nuclear
P2 S
HG140
HG188
HG164
HG122
G2955
-SIRT1
-β-Catenin
-Lamin A
-Progerin
-Lamin C
-β-actin
D
Relative level of
Nuclear matrix-bound SIRT1
Healthy
HGPS
Non-progeria
E
Relative increase of deacetylase
activity of nhSIRT1
NE Lysate
Cytoplasmic
Nuclear matrix
F
Nicotinamide
Resveratrol
shLamin A
shSIRT1
Flag-P53
AcK
P53
SIRT1
Lamin A
G
Veh
RSV_1
RSV_10
-Sirt1
-β-actin
Z+/+
Z-/-
H
Percent level of NM-Sirt1
Zmpste24+/+
Zmpste24-/-

FIG. 7

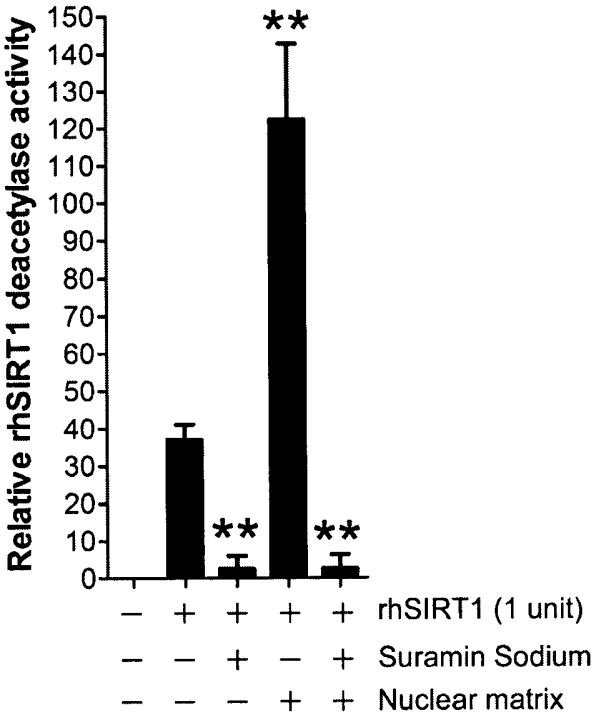
Relative rhSIRT1 deacetylase activity
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
**
**
**
– + + + + rhSIRT1 (1 unit)
– – + – + Suramin Sodium
– – – + + Nuclear matrix

FIG. 7I

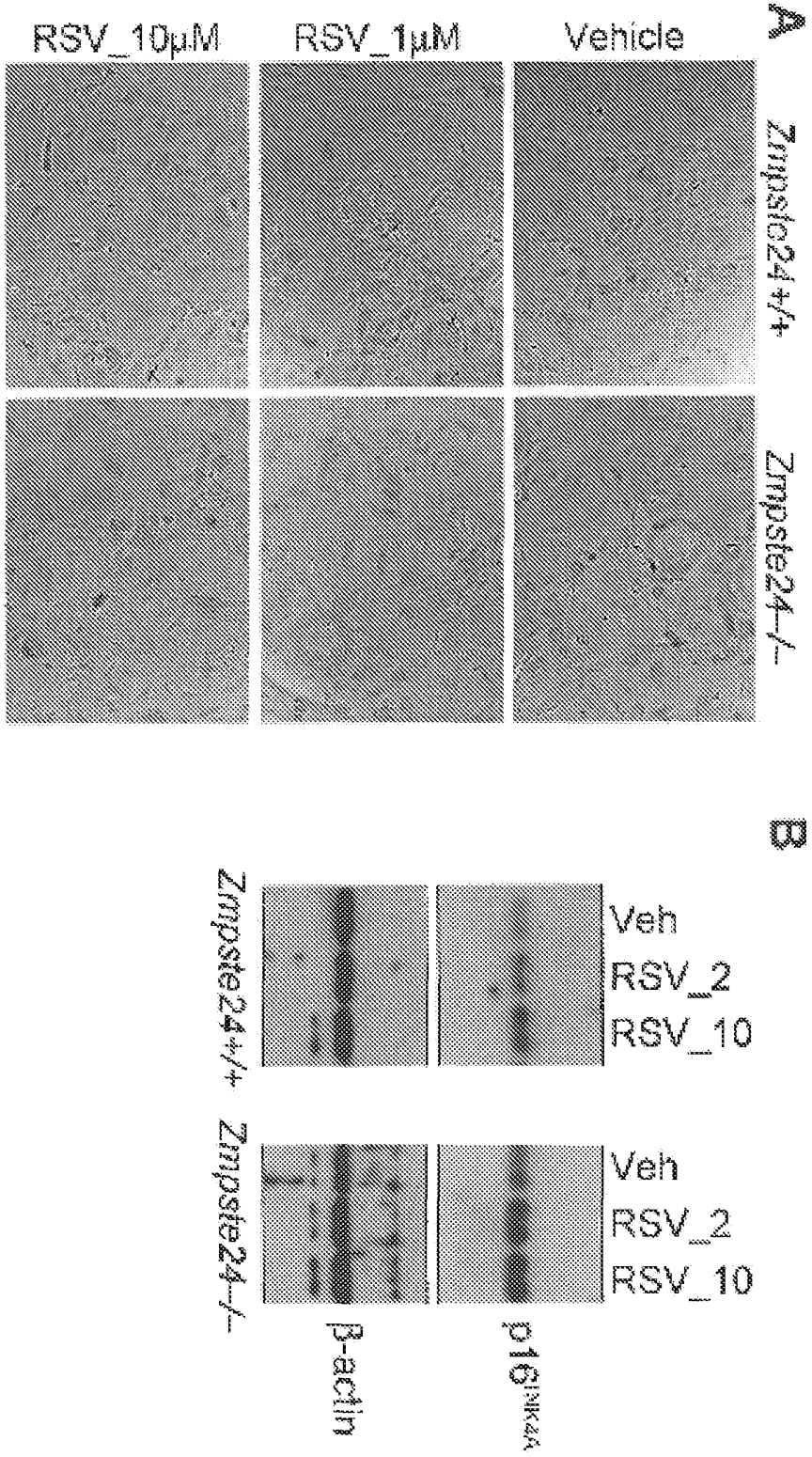
A
RSV_10μM
RSV_1μM
Vehicle
Zmpste24+/+
Zmpste24−/−
B
Zmpste24+/+
Zmpste24−/−
Veh
RSV_2
RSV_10
Veh
RSV_2
RSV_10
β-actin
p16INK4A

FIG. 8

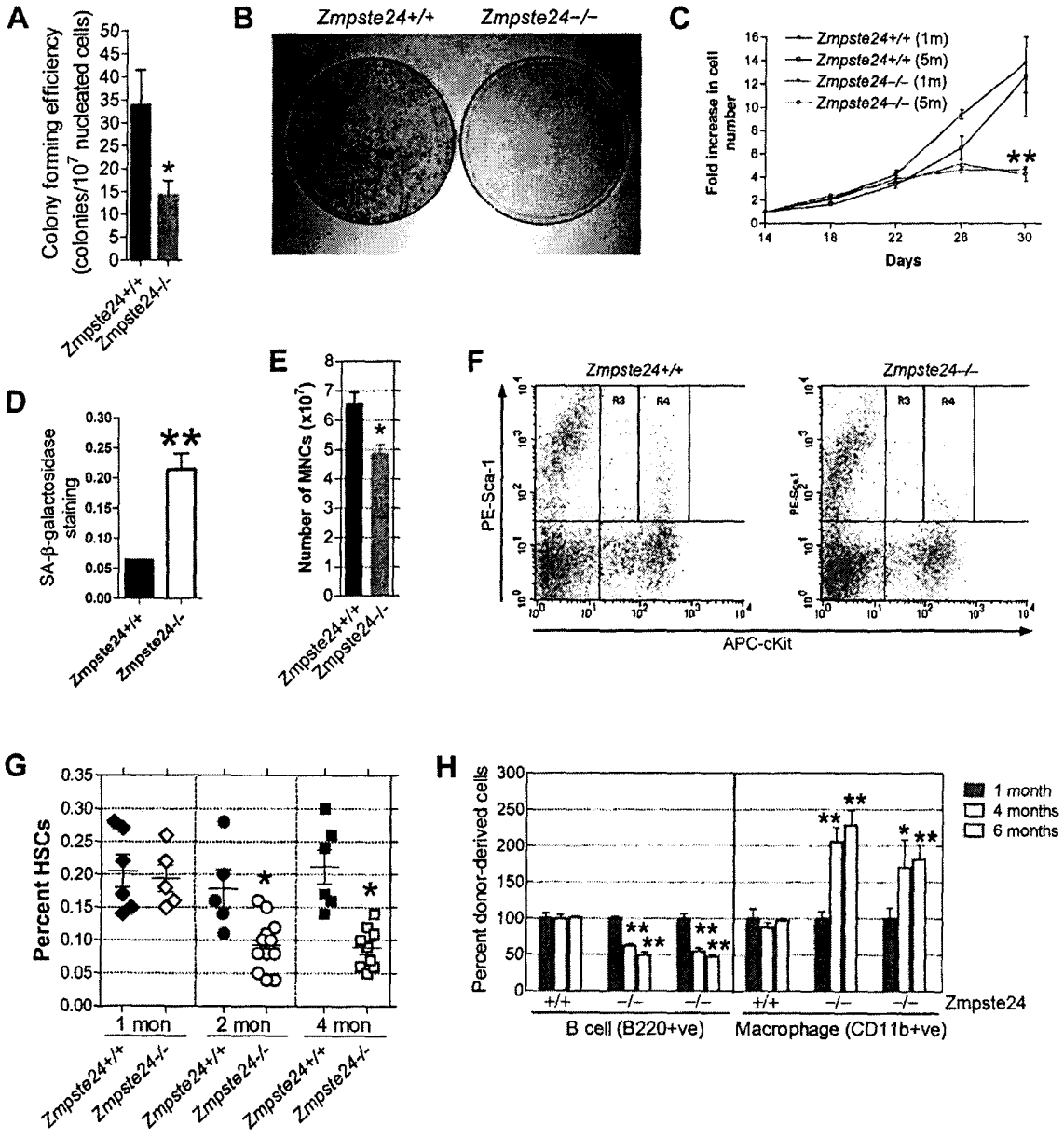
A
Colony forming efficiency (colonies/10^7 nucleated cells)
Zmpste24+/+
Zmpste24-/-
B
Zmpste24+/+
Zmpste24-/-
C
Zmpste24+/+ (1m)
Zmpste24+/+ (5m)
Zmpste24-/- (1m)
Zmpste24-/- (5m)
Fold increase in cell number
Days
D
SA-β-galactosidase staining
E
Number of MNCs (x10^7)
F
Zmpste24+/+
Zmpste24-/-
PE-Sca-1
APC-cKit
R3
R4
G
Percent HSCs
1 mon
2 mon
4 mon
H
Percent donor-derived cells
1 month
4 months
6 months
Zmpste24
B cell (B220+ve)
Macrophage (CD11b+ve)

FIG. 9

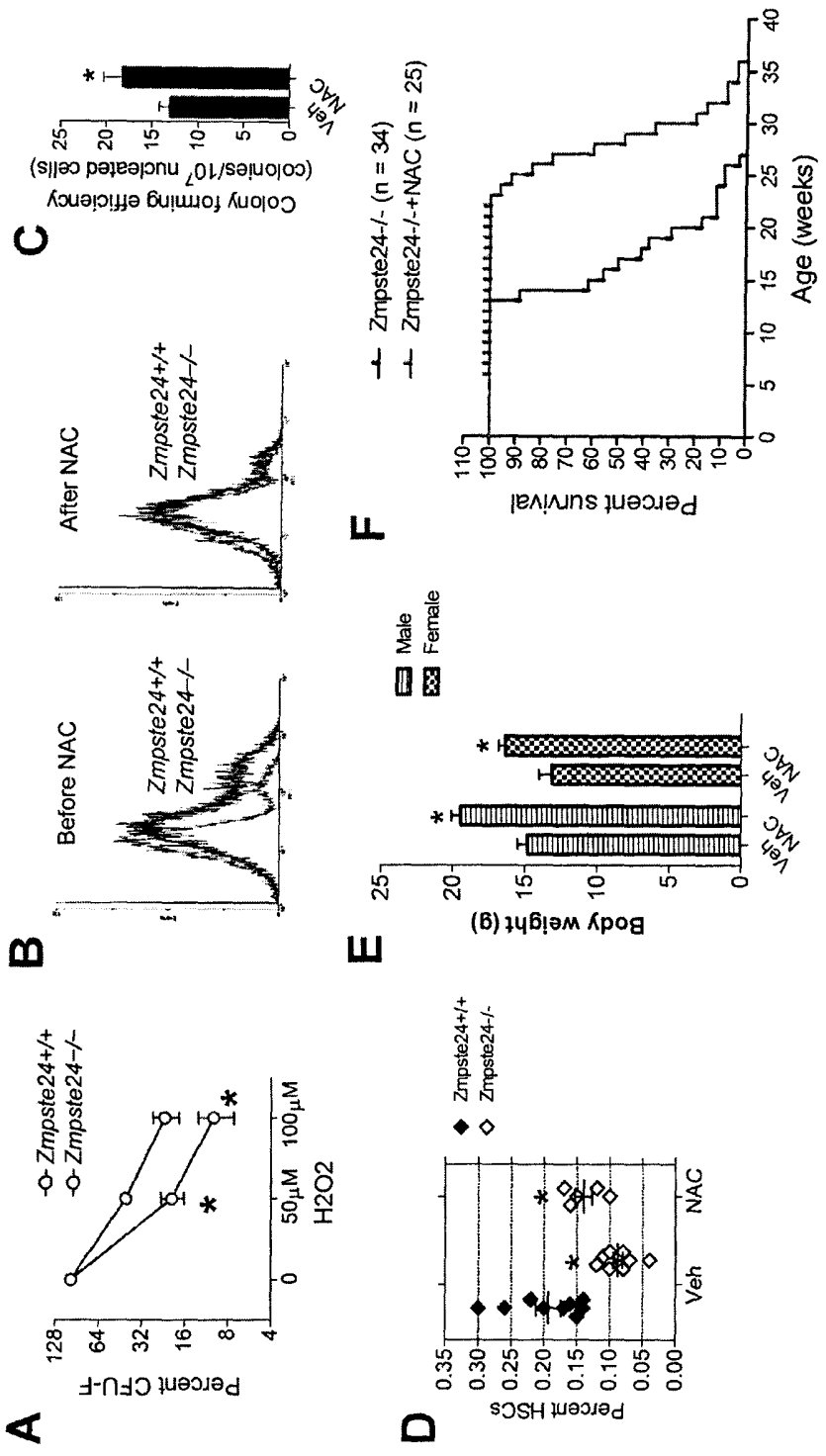
A
Zmpste24+/+
Zmpste24−/−
Percent CFU-F
128
64
32
16
8
4
0
50μM
100μM
H2O2
B
Before NAC
After NAC
Zmpste24+/+
Zmpste24−/−
C
Colony forming efficiency
(colonies/10^7 nucleated cells)
25
20
15
10
5
0
Veh
NAC
D
Zmpste24+/+
Zmpste24-/-
Percent HSCs
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Veh
NAC
E
Male
Female
Body weight (g)
25
20
15
10
5
0
Veh
NAC
Veh
NAC
F
Zmpste24-/- (n = 34)
Zmpste24-/-+NAC (n = 25)
Percent survival
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
Age (weeks)

FIG. 10

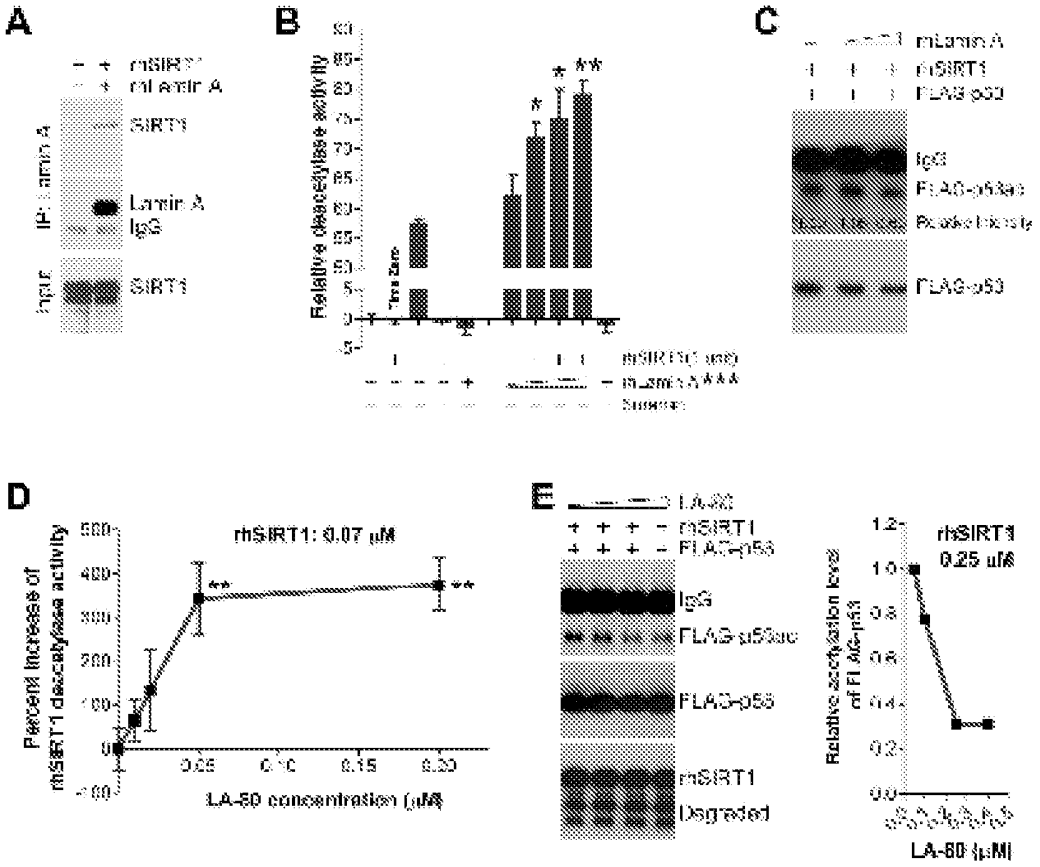
A
B
C
D
E
rhSIRT1
rLamin A
SIRT1
Lamin A
IgG
Relative deacetylase activity
Time Zero
rhSIRT1 (1 unit)
rLamin A
Substrate
rLamin A
rhSIRT1
FLAG-p53
IgG
FLAG-p53ac
Protein blot by
FLAG-p53
rhSIRT1: 0.07 μM
Percent increase of rhSIRT1 deacetylase activity
LA-50 concentration (μM)
LA-50
rhSIRT1
FLAG-p53
IgG
FLAG-p53ac
FLAG-p53
rhSIRT1
Degraded
rhSIRT1
0.25 u/l
Relative acetylation level of FLAG-p53
LA-50 (μM)

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/086954

A. CLASSIFICATION OF SUBJECT MATTER

See the extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNKI, CNABS, VEN, NCBI, ISI-WEB OF KNOWLEDGE, GOOGLE SCHOLAR: lamin A, SIRT1, resveratrol, deacetylase, activator, inhibitor, screen, binding affinity, sirtuin, LMNA, modulate aging, cancer, tumor, carcinoma, malignancy

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Villalba, J.M. et al. Sirtuin activators and inhibitors. Biofactors. September 2012, vol.38, no.5, pages 349-359 See the whole document, especially the abstract	1, 2, 4, 11, 12, 14-17
X	Kim, E.J. et al. SIRT1: roles in aging and cancer. BMB REPORTS. 2008, vol.41, no.11, pages 751-756 See the whole document, especially the abstract, pages 752 and 753	1, 2, 4, 11, 12, 14-17

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 February 2014 (10.02.2014)	**20 Feb. 2014 (20.02.2014)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer TIAN, Yuan Telephone No. (86-10)62411047

Form PCT/ISA /210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/086954

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Borra, M.T.et al. Mechanism of Human SIRT1 Activation by Resveratrol. JOURNAL OF BIOLOGICAL CHEMISTRY. 2005, vol.280, no.17, pages 17187-17195 See the whole document, especially the abstract and page 17194	1, 2, 4
X	Yang, Hongying et al. Design and synthesis of compounds that extend yeast replicative lifespan. Aging Cell. February 2007, vol.6, no.1, pages 35-43 See the whole document, especially the abstract and page 37	18
A		19-21
PX	Ghosh Shrestha et al. Resveratrol activates SIRT1 in a Lamin A-dependent manner. Cell Cycle.March 2013, vol.12. no.6, pages 872-876 See the whole document	1-18
PX	Liu, Baohua et al. Resveratrol Rescues SIRT1-Dependent Adult Stem Cell Decline and Alleviates Progeroid Features in Laminopathy-Based Progeria. Cell Metabolism. December 2012, vol. 16, no.6, pages 738-750 See the whole document	1-17
PX	Liu, Baohua et al. Activation of SIRT1 by Resveratrol requires lamin A. AGING February 2013, vol.5, no.2, pages 94-95 See the whole document	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/086954

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item item1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of:

   a. a sequence listing filed or furnished

      ☐ on paper

      ☒ in electronic form

   b. time of filing or furnishing

      ☐ contained in the applicantion as filed

      ☐ filed together with the application in electronic form

      ☒ furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The sequence listing furnished subsequently to this Authority for the purposes of search is not described in the original disclosure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/086954

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 11-17
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 11-17 are directed to a method of treatment of human/animal body by therapy (Rules 39.1 (iv) PCT), however, the search has been carried out and based on the alleged effects of the compositions.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/086954

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/16 (2006.01) i
A61P 3/00 (2006.01) i
A61P 39/00 (2006.01) i
A61P 35/00 (2006.01) i